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[US/US]; 330 East 38th Street, Apt. 35B, New York, NY 10016 (US). **WISNIEWSKI, Thomas** [US/US]; 86 Ward Avenue, Staten Island, NC 10304 (US). **SIGURDSSON, Einar, M.** [IS/US]; 120 Suffolk Street, Apt. 2B, New York, NY 10002 (US).

(54) Title: SYNTHETIC IMMUNOGENIC BUT NON-DEPOSIT-FORMING POLYPEPTIDES AND PEPTIDES HOMOLOGOUS TO AMYLOID β, PRION PROTEIN, AMYLIN, α-SYNUCLEIN, OR POLYGLUTAMINE REPEATS FOR INDUCTION OF AN IMMUNE RESPONSE THERETO

—■— Aβ1-30-NH₂
—●— K6Aβ1-30-NH₂
—▼— Aβ1-42
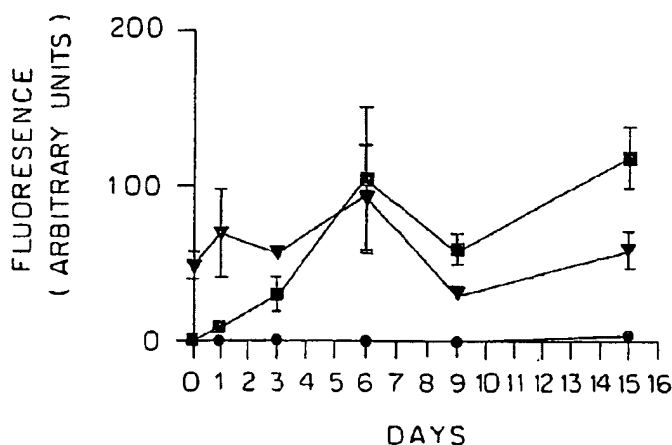
(57) Abstract: The present invention relates to immunogenic but non-depositing-forming polypeptides or peptides homologous to amyloid β, prion, amylin or α-synuclein which can be used alone or conjugated to an immunostimulatory molecule in an immunizing composition for inducing an immune response to amyloid β peptides and amyloid deposits, to prion · protein and prion deposits, to amylin and amylin deposits, to α-synuclein and deposits containing α-synuclein, or to polyglutamine repeats and deposits of proteins containing polyglutamine repeats. Described are also antibodies directed against such peptides, their generation, and their use in methods of passive immunization to such peptides and deposits.

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SYNTHETIC IMMUNOGENIC BUT NON-DEPOSIT-FORMING POLYPEPTIDES AND PEPTIDES HOMOLOGOUS TO AMYLOID β, PRION PROTEIN, AMYLIN, α-SYNUCLEIN, OR POLYGLUTAMINE REPEATS FOR INDUCTION OF AN IMMUNE RESPONSE THERETO

[0001] This application claims the priority under 35 U.S.C.

119(e) of U.S. Provisional Application No. 60/331,801, filed

November 21, 2001, which is hereby incorporated by reference in

its entirety.

GOVERNMENT LICENSE RIGHTS

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invention and the right in limited circumstances to require the

patent owner to license others on reasonable terms as provided

for by the terms of each respective grant listed above.

BACKGROUND OF THE INVENTION

Field of the Invention

[0003] The present invention relates to the field of amyloid β

peptides, prion protein, amylin, α-synuclein, and polyglutamine

repeats and methods for inducing an immune response to amyloid β

peptides and amyloid deposits, prion proteins and deposits,
amylin and amylin fibrils, filaments and fibrils containing α-
synuclein, or protein aggregates containing polyglutamine
repeats. The invention also relates to a method of treating or
preventing amyloid plaque forming diseases or amyloidosis by the
use of a synthetic immunogenic but not deposit forming
polypeptide homologous to the protein which forms the amyloid
plaque.

Description of the Background Art

Amyloid β

[0004] Alzheimer's disease (AD) is the most common form of
late-life dementia in adults (Soto et al., 1994), constituting
the fourth leading cause of death in the United States.
Approximately 10% of the population over 65 years old is affected
by this progressive degenerative disorder that is characterized
by memory loss, confusion and a variety of cognitive
disabilities. Neuropathologically, AD is characterized by four
major lesions: a) intraneuronal, cytoplasmic deposits of
neurofibrillary tangles (NFT), b) parenchymal amyloid deposits
called neuritic plaques, c) cerebrovascular amyloidosis, and d)
synaptic and neuronal loss. One of the key events in AD is the
deposition of amyloid as insoluble fibrous masses
(amyloidogenesis) resulting in extracellular neuritic plaques and

deposits around the walls of cerebral blood vessels. The major

constituent of the neuritic plaques and congophilic angiopathy is

amyloid β (Aβ), although these deposits also contain other

proteins such as glycosaminoglycans and apolipoproteins.

[0005] Aβ is a 4.1-4.3 kDa hydrophobic peptide that is

codified in chromosome 21 as part of a much longer amyloid

precursor protein APP (Muller-Hill et al., 1989). The APP starts

with a leader sequence (signal peptide), followed by a cysteine-

rich region, an acidic-rich domain, a protease inhibitor motif, a

putative N-glycosylated region, a transmembrane domain, and

finally a small cytoplasmic region. The Aβ sequence begins close

to the membrane on the extracellular side and ends within the

membrane. Two-thirds of Aβ faces the extracellular space, and

the other third is embedded in the membrane (Kang et al., 1987

and Dyrks et al., 1988). Several lines of evidence suggest that

amyloid may play a central role in the early pathogenesis of AD.

[0006] Evidence that amyloid may play an important role in the

early pathogenesis of AD comes primarily from studies of

individuals affected by the familial form of AD (FAD) or by

Down's syndrome. Down's syndrome patients have three copies of

the APP gene and develop AD neuropathology at an early age

(Wisniewski et al., 1985). Genetic analysis of families with

hereditary AD revealed mutations in chromosome 21, near or within

the Aβ sequence (Forsell et al., 1995), in addition to mutations

within the presenilin 1 and 2 genes. Moreover, it was reported that transgenic mice expressing high levels of human mutant APP progressively develop amyloidosis in brain (Games et al., 1995). These findings appear to implicate amyloidogenesis in the pathophysiology of AD. In addition, Aβ fibrils are toxic in neuronal culture (Yankner et al., 1989) and to some extent when injected into animal brains (Sigurdsson et al., 1996 and 1997).

[0007] Furthermore, several other pieces of evidence suggest that the deposition of Aβ is a central triggering event in the pathogenesis of AD, which leads subsequently to NFT formation and neuronal loss. The amyloid deposits in AD share a number of properties with all the other cerebral amyloidoses, such as the prion related amyloidoses, as well as the systemic amyloidoses. These characteristics are: 1) being relatively insoluble; 2) having a high β-sheet secondary structure, which is associated with a tendency to aggregate or polymerize; 3) ultrastructurally, the deposits are mainly fibrillary; 4) presence of certain amyloid-associated proteins such as amyloid P component, proteoglycans and apolipoproteins; 5) deposits show a characteristic apple-green birefringence when viewed under polarized light after Congo red staining.

[0008] The same peptide that forms amyloid deposits in AD brain was also found in a soluble form (sAβ) normally circulating in the human body fluids (Seubert et al., 1992 and Shoji et al.,

1992). Zlokovic et al. (1994), reported that the blood-brain barrier (BBB) has the capability to control cerebrovascular sequestration and transport of circulating sAβ, and that the transport of the sAβ across the BBB was significantly increased when sAβ was perfused in guinea pigs as a complex with apolipoprotein J (apoJ). The sAβ-apoJ complex was found in normal cerebrospinal fluid (CSF; Ghiso et al., 1994) and in *vivo* studies indicated that sAβ is transported with apoJ as a component of the high density lipoproteins (HDL) in normal human plasma (Koudinov et al., 1994). It was also reported by Zlokovic et al. (1996), that the transport of sAβ across the BBB was almost abolished when the apoJ receptor gp330 was blocked. It is believed that the conversion of sAβ to insoluble fibrils is initiated by a conformational modification of the 2-3 amino acid longer soluble form. It has been suggested that the amyloid formation is a nucleation-dependent phenomena in which the initial insoluble "seed" allows the selective deposition of amyloid (Jarrett et al., 1993).

[0009] Peptides containing the sequence 1-40 or 1-42 of Aβ and shorter derivatives can form amyloid-like fibrils in the absence of other protein (Soto et al., 1994), suggesting that the potential to form amyloid resides mainly in the structure of Aβ. The relation between the primary structure of Aβ and its ability to form amyloid-like fibrils was analyzed by altering the

sequence of the peptide. Substitution of hydrophilic residues

for hydrophobic ones in the internal Aβ hydrophobic regions

(amino acids 17-21) impaired fibril formation (Hilbich et al.,

1992), suggesting that Aβ assembly is partially driven by

hydrophobic interactions. Indeed, larger Aβ peptides (Aβ1-42/43)

comprising two or three additional hydrophobic C-terminal

residues are more amyloidogenic (Jarrett et al., 1993).

Secondly, the conformation adopted by Aβ peptides is crucial in

amyloid formation. Aβ peptides incubated at different pH,

concentrations and solvents can have either a mainly α-helical,

random coil, or a β-sheet secondary structure (Barrow et al.,

1992; Burdick et al., 1992 and Zagorski et al., 1992). The Aβ

peptide with α-helical or random coil structure aggregates

slowly; Aβ with β-sheet conformation aggregates rapidly (Zagorski

et al., 1992; Soto et al., 1995 and Soto et al., 1996). The

importance of hydrophobicity and β-sheet secondary structure on

amyloid formation also is suggested by comparison of the sequence

of other amyloidogenic proteins.

[0010] Analysis of Aβ aggregation by turbidity measurements

indicates that the length of the C-terminal domain of Aβ

influences the rate of Aβ assembly by accelerating nucleus

formation (Jarrett et al., 1993). Thus, the C-terminal domain of

Aβ may regulate fibrillogenesis. However, *in vitro* modulators of

Aβ amyloid formation, such as metal cations (Zn, Al) (Bush et

al., 1994 and Exley et al., 1993) heparin sulfate proteoglycans,

and apoliprotein E (Strittmatter et al., 1993) interact with the

12-28 region of Aβ. Moreover, mutations in the APP gene within

the N-terminal Aβ domain yield analogs more fibrillogenic (Soto

et al., 1995 and Wisniewski et al., 1991). Finally, while the C-

terminal domain of Aβ invariably adopts a β-strand structure in

aqueous solutions, environmental parameters determine the

existence of alternative conformation in the Aβ N-terminal domain

(Barrow et al., 1992; Soto et al., 1995 and Burdick et al.,

1992). Therefore, the N-terminus may be a potential target site

for inhibition of the initial random coil to β-sheet

conformational change.

[0011] The emerging picture from studies with synthetic

peptides is that Aβ amyloid formation is dependent on hydrophobic

interactions of Aβ peptides adopting an antiparallel β-sheet

conformation and that both the N- and C-terminal domains are

important for amyloid formation. The basic unit of fibril

formation appears to be the conformer adopting an antiparallel β-

sheet composed of strands involving the regions 10-24 and 29-

40/42 of the peptide (Soto et al., 1994). Amyloid formation

proceeds by intermolecular interactions between the β-strands of

several monomers to form an oligomeric β-sheet structure

precursor of the fibrillar β-cross conformation. Wood et al.,

(1995) reported the insertion of aggregation-blocking prolines into amyloid proteins and peptides to prevent aggregation of such proteins and peptides. In this manner, the authors suggest that novel proteins can be designed to avoid the problem of aggregation as a barrier to their production without affecting the structure or function of the native protein. Thus, Wood et al. seek to produce novel proteins that would not aggregate during recombinant protein production and purification by inserting aggregation/blocking prolines into these novel peptides.

[0012] To date there is no cure or effective therapy for reducing a patient's amyloid burden or preventing amyloid deposition in AD, and even the unequivocal diagnosis of AD can only be made after postmortem examination of brain tissues for the hallmark neurofibrillary tangles (NFT) and neuritic plaques. However, there are an increasing number of publications outlining strategies for the treatment of Alzheimer's disease. Amyloid-related therapeutic strategies include the use of compounds that affect processing of the amyloid-β precursor protein (APP; Dovey et al., 2001), that interfere with fibril formation or that promote fibril disassembly (Soto et al., 1998; Sigurdsson et al., 2000; and Findeis, 2000).

[0013] Heparin sulfate (glycosoaminoglycan) or the heparin sulfate proteoglycan, perlecan, has been identified as a component of all amyloids and has also been implicated in the earliest stages of inflammation-associated amyloid induction. Kisilevsky et al. (1995) describes the use of low molecular weight (135 - 1,000 Da) anionic sulfonate or sulfate compounds that interfere with the interaction of heparin sulfate with the inflammation-associated amyloid precursor and the β-peptide of AD. Heparin sulfate specifically influences the soluble amyloid precursor (SAA2) to adopt an increased β-sheet structure characteristic of the protein-folding pattern of amyloids. These anionic sulfonate or sulfate compounds were shown to inhibit heparin-accelerated Alzheimer's Aβ fibril formation and were able to disassemble preformed fibrils *in vitro* as monitored by electron micrography. Moreover, when administered orally at relatively high concentrations (20 or 50 mM), these compounds substantially arrested murine splenic inflammation-associated amyloid progression *in vivo* in acute and chronic models. However, the most potent compound, poly-(vinylsulfonate), was acutely toxic.

[0014] Anthracycline 4'-iodo-4'-deoxy-doxorubicin (IDOX) has been observed clinically to induce amyloid resorption in patients with immunoglobin light chain amyloidosis (AL). Merlini et al. (1995), elucidated its mechanism of action. IDOX was found to

bind strongly via hydrophobic interactions to two distinct binding sites (Scatchard analysis) in five different tested amyloid fibrils, inhibiting fibrillogenesis and the subsequent formation of amyloid deposits *in vitro*. Preincubation of IDOX with amyloid enhancing factor (AEF) also reduced the formation of amyloid deposits. Specific targeting of IDOX to amyloid deposits *in vivo* was confirmed in an acute murine model. This binding is distinct from heparin sulfate binding as removal of the glycosaminoglycans from extracted amyloid fibrils with heparinases did not modify IDOX binding. The common structural feature of all amyloids is a β-pleated sheet conformation. However, IDOX does not bind native amyloid precursor light chains which suggests that the β-pleated sheet backbone alone is not sufficient to form the optimal structure for IDOX binding, and that it is the fibril cross-β-sheet quaternary structure that is required for maximal IDOX binding. It has been found that the amount of IDOX extracted from spleens is correlated with amyloid load and not circulating serum precursor amyloid levels. IDOX, however, is also extremely toxic.

[0015] The regulation and processing of amyloid precursor protein (APP) via inhibition or modulation of phosphorylation of APP control proteins has also been investigated in U.S. Patent 5,385,915 and WO 9427603. Modulating proteolytic processing of APP to nucleating forms of AD has also been examined in AU

9338358 and EP569777. WO 95/046477 discloses synthetic peptides
of composition X-X-N-X coupled to a carrier, where X is a
cationic amino acid and N is a neutral amino acid, which inhibit
Aβ binding to glycosoaminoglycan. Peptides containing Alzheimer's
Aβ sequences that inhibit the coupling of α-1-antichymotrypsin
and Aβ are disclosed in WO 92/03474.

[0016] From experiments conducted at the laboratory of the
present inventors, WO 96/39834 discloses that peptides capable of
interacting with a hydrophobic portion on a protein or peptide,
such as Aβ, involved in amyloid-like deposit formation can be
used to inhibit and structurally block the abnormal folding of
such proteins and peptides into amyloid or amyloid-like deposits.
The peptides which block abnormal folding of Aβ into amyloid
deposits have a hydrophobic portion containing β-sheet breaking
amino acid residue(s), such as proline, that reduces the
propensity of the peptide for adopting a β-sheet conformation.
The laboratory of the present inventors, in later reports, have
demonstrated that LeuProPhePheAsp (SEQ ID NO:14), a non-
amyloidogenic peptide with sequence homology to Aβ blocks fibril
formation (Soto et al., 1998), and induces in vivo disassembly of
fibrillar Aβ deposits (Sigurdsson et al., 2000).

[0017] Recently, the coupling of lysine residues to peptides
was proposed by Pallitto et al. (1999), in the design of anti-β
sheet peptides or Aβ fibrillogenesis inhibitors that have an Aβ-

binding recognition sequence and a hexameric lysine aggregation disrupting element.

[0018] *In vitro* studies have shown that monoclonal antibodies raised against the N-terminal region of Aβ can disaggregate Aβ fibrils, maintain Aβ solubility, and prevent Aβ toxicity in cell culture (Solomon et al., 1996 and 1997).

[0019] WO 96/25435 discloses the potential for using a monoclonal antibody, which is end-specific for the free C-terminus of the Aβ1-42 peptide, but not for the Aβ1-43 peptide, in preventing the aggregation of Aβ1-42. The administration of such an Aβ end-specific monoclonal antibody is further disclosed to interact with the free C-terminal residue of Aβ1-42, thereby interfering with and disrupting aggregation that may be pathogenic in AD.

[0020] WO 98/44955 takes a different approach to avoiding the problems associated with repeated administration of pharmacological agent and discloses a method for preventing the onset of Alzheimer's Disease or for inhibiting progression of Alzheimer's Disease through the stable ectopic expression in the brain of recombinant antibodies end-specific for amyloid-β peptides.

[0021] Recently, Schenk et al. (1999) demonstrated that immunization with amyloid-β attenuated Alzheimer's disease-like pathology in PDAPP transgenic mice serving as an animal model for

amyloid-β deposition and Alzheimer's disease-like

neuropathologies. They reported that immunization of young

animals prior to the onset of Alzheimer's disease-type

neuropathologies essentially prevented the development of β-

amyloid plaque formation, neuritic dystrophy and astrogliosis,

whereas treatment in older animals after the onset of Alzheimer's

disease-type neuropathologies was observed to reduce the extent

and progression of these neuropathologies. This effect is

thought to be mediated by antibodies, since peripherally

administered antibodies against Aβ have been shown to reduce

brain parenchymal amyloid burden (Bard et al., 2000). In

addition, intranasal immunization with freshly solubilized Aβ1-40

reduces cerebral amyloid burden (Weiner et al., 2000). Two

recent studies demonstrated that a vaccination-induced reduction

in brain amyloid deposits resulted in cognitive improvements

(Morgan et al., 2000; Janus et al., 2000).

[0022] Although the results reported by Schenk et al. provides

promise for using immunomodulation as a general approach to treat

Alzheimer's disease, immunization with intact amyloid-β according

to Schenk et al. presents problems that make it inappropriate for

human use. First, Schenk et al's experiments used transgenic

mice which express a mutated human protein that is foreign to

them and that has no physiological function in mice (the mouse

and human Aβ peptide sequences are significantly different).

However, in humans, the precursor protein (βAPP) is an endogenous

protein that has a normal function. Hence, using this approach

in humans with a human Aβ peptide may well lead to development of

an autoimmune disorder or disease that could make matters worse

not better. Second, Martel et al. (1996) and the present

inventors have results which demonstrate that Aβ peptides, Aβ1-42

and Aβ1-40, can cross the blood brain barrier in experimental

animals. Therefore, in humans, it is expected that Aβ1-42, which

is used for immunization in Schenk et al., can cross the blood

brain barrier and co-deposit on any existing amyloid plaques

leading to increased toxicity, and may actually promote plaque

formation. This has not been a problem in the PDAPP transgenic

mouse model for AD because human Aβ1-42 is less toxic for the

mouse; even with massive deposition of human Aβ1-42, none of the

transgenic mice show significant neuronal loss. Thirdly, Schenk

et al. use a toxic adjuvant to induce an immune response.

Prion

[0023] From a mechanistic point of view, the best understood

of the conformational disorders are the prion related diseases

(or prionoses). The etiology of these diseases is the conversion

of the normal prion protein, PrP^c, into its infectious and

pathogenic form, PrP^{sc} (Prusiner et al., 1998; Horwich and

Weismann, 1997). PrP^C and PrP^{Sc} are thought to differ only in their conformation, with PrP^{Sc} having a greater ß-sheet content.

[0024]   The first of these disorders to be described was scrapie, a disease of sheep recognized for over 250 years. This illness manifested by hyper-excitability, itching and ataxia, leads to paralysis and death. It is called scrapie because of the tendency of affected animals to rub against the fences of their pens in order to stay upright, reflecting their cerebellar dysfunction. The transmission of this disease was demonstrated first in 1943 when a population of Scottish sheep was accidentally inoculated against a common virus using a formalin extract of lymphoid tissue from an animal with scrapie (Gordon, 1946). After an incubation period of two years about 10% of the inoculated animals developed scrapie.

[0025]   The first human prionosis was described some years later and is called kuru (Gajdusek and Zigas, 1957; Gajdusek and Zigas, 1959). This is an illness of the Fore people living in the highlands of New Guinea, that is thought to be linked to ritualistic cannibalism. Presumably this illness originated with the accidental consumption of an initial patient with sporadic Creutzfeldt-Jacob disease (CJD). Kuru was once the major cause of death among Fore women; however, the disease has virtually disappeared with the end of cannibalistic rituals. Rather similar to scrapie, patients clinically present with difficulty

walking and develop progressive signs of cerebellar dysfunction. Death occurs approximately 1 year following onset of symptoms. The neuropathology of kuru, in common with all prionoses to a variable extent, includes widespread spongiform change and astrocytosis, as well as neuronal loss affecting the cerebral hemispheres and cerebellum. In about 70% of cases, amyloid plaques are found, with amyloid deposition being a common but not invariable accompaniment of the prionoses. It was Gajdusek's detailed description of this illness that lead Hadlow to suggest that kuru might be the human representation of scrapie (Hadlow, 1959). This in turn suggested to Gajdusek and his team to test whether kuru was also transmissible. They first showed kuru was transmissible to chimpanzees, after a long incubation, in 1966 (Gajdusek et al., 1966); this work led to Gajdusek being awarded the Nobel Prize in 1976.

[0026] Other human prionoses include Gerstamann-Sträussler-Scheinker disease (GSS), described in a large kindred in 1936 (Gerstmann et al., 1936). This illness presents with a slowly progressive limb and truncal ataxia, as well as dementia, with death occurring from 6 to 10 years following presentation. The pattern of inheritance is autosomal dominant; it is now known that all cases of GSS are associated with mutations of the PrP gene. The neuropathology of GSS is remarkable in that there is extensive and invariable amyloid deposition, in addition to the

typical spongiform change, gliosis and neuronal loss.
Interestingly, in several kindreds of GSS, extensive
neurofibrillary tangle (NFT) formation is found (Ghetti et al.,
1994). NFTs are an essential feature of AD. Another variation
of autosomal dominantly inherited human prionosis has been termed
prion protein-congophilic angiopathy (PrP-CAA), which is
characterized by cerebral vessel PrP amyloid deposition and the
presence of NFT (Ghetti et al., 1996). CAA is also an essential
feature of AD. Both these variants of prionoses further link the
pathogenesis of AD and the prion related diseases.

[0027] Creutzfeldt-Jacob disease (CJD) was initially described
by Jacob in 1921 (Jacob, 1921); ironically, the case reported by
Creutzfeldt a year earlier is probably unrelated to the disease
which carries his name. Clinically CJD is characterized by a
rapidly progressive dementia, associated with myoclonic jerks, as
well as a variable constellation of pyramidal, extrapyramidal and
cerebellar signs. The EEG typically shows distinctive changes of
high voltage, slow (1 to 2Hz) and sharp wave complexes on an
increasingly slow and low-voltage background. CJD is found
throughout the world with an incidence of about 1 per million.
In addition to extensive cortical spongiosis, gliosis and
neuronal loss, 10% of CJD cases have amyloid plaques (Prusiner et
al., 1998).

[0028] Fatal familial insomnia (FFI) is a disorder presenting with intractable insomnia, dysautonomia, a variety of endocrine abnormalities and motor paralysis (Medori et al., 1992). Neuropathologically, there is marked atrophy of the anterior ventral and mediodorsal thalamic nuclei, due to neuronal loss and gliosis. Unlike other prionoses, spongiform change can be a minor feature or be absent altogether. All patients with FFI have a missense mutation at codon 178 of the PrP gene where Asn is replaced by Asp, coupled with a Met at the polymorphic codon 129 (Goldfarb et al., 1992). The somewhat divergent clinical and neuropathological features of FFI, in comparison to other human prionoses, highlight the wide spectrum of disease associated with PrP dysfunction and suggests that there may be other human illnesses which have yet to be recognized as prionoses.

[0029] There has been a recent epidemic of a new prionosis, bovine spongiform encephalopathy (BSE), that has led to more than 160,000 cattle deaths in the UK (Collinge, 1997). This new disease is thought to be caused by meat and bone meal dietary supplements to cattle that were contaminated with scrapie infected sheep and other cattle with BSE. Some evidence suggests that BSE also has led to a new type of CJD, called new variant CJD (vCJD) (Collinge et al., 1996a). The first cases of vCJD were reported in 1995, when two cases of CJD were found in 2 British teenagers (Bateman et al., 1995; Britton et al., 1995).

Only 4 cases of sporadic CJD have been reported previously among teenagers; the peak onset of sporadic CJD being between ages 60 to 65 years. In addition to the early age, these cases had distinctive neuropathology that included so-called "florid" amyloid plaques which are reminiscent of kuru associated PrP amyloid plaques (Collee and Bradley, 1997; Will et al., 1997). Since the original reports, there have been 14 cases with these distinctive features; all were in the UK except for one French case. The emergence of vCJD has raised the specter of an epidemic of prion related disease among the British population similar to that of BSE in cattle.

[0030] Highly divergent hypotheses have been put forward regarding the etiology of the prionoses, including that they consist of nucleic acid only, protein only, are lacking both protein and nucleic acid or are a polysaccharide. The most widely accepted hypothesis, first put forward by Griffith (Griffith, 1967) and more explicitly by Pruisner is the "protein only" hypothesis (Prusiner et al., 1998). Pruisner introduced the term "prion" to indicate that scrapie is related to a *proteinous infectious particle* (Prusiner, 1982). This hypothesis was initially greeted with great scepticism in the scientific community; now it represents the current dogma with Dr. Pruisner being honored with the 1998 Noble Prize for Science. This hypothesis suggests that prions contain no nucleic acid and are

referred to as PrP^{Sc}. The latter represents a conformationally modified form of a normal cellular PrP^C, which is a normal host protein found on the surface of many cells, in particular neurons. PrP^{Sc} when introduced into normal, healthy cells causes the conversion of PrP^C into PrP^{Sc}, initiating a self-perpetuating vicious cycle (Prusiner et al., 1998).

[0031] Other hypotheses for prion have included the "virino hypothesis" (Weissmann, 1996). Here it is suggested that the infectious agent consists of a nucleic acid with host derived PrP^{Sc} serving as a coat. The latter would explain the lack of an immunological and inflammatory response, while the presence of a nucleic acid provides an explanation for the numerous strains of scrapie, each with distinctive features. Other investigators have also suggested that the scrapie agent is a conventional virus with highly atypical properties. However, despite extensive searches, no nucleic acid associated with prion infection has been detected so far. The unusual nature of the scrapie agent first became apparent during the early transmission studies, where it first was found that infectivity was filterable, consistent with the presence of a virus, but differing from viruses in that formalin treatment did not abolish this infectivity (Gordon, 1946). Later Alper et al. showed that the scrapie agent was resistant to inactivation by UV at 254nm unlike nucleic acid (Alper et al., 1967), but sensitive to irradiation

at 237nm, suggestive of the presence of a protein (Latarjet et al., 1970). The possibility that a protein was involved was furthered by the pioneering work of Pruisner et al. with the biochemical partial purification of infectious activity (Prusiner, 1982). These workers reported a 1000 fold increase in infectivity compared to homogenates of infectious brain by a series of steps involving polyethylene glycol precipitation, nuclease digestion, partial proteinase K (PK) digestion and density gradient centrifugation. The enriched infectious activity was inactivitated or reduced by extensive PK digestion, diethyl pyrocarbonate, urea, chaotropes, phenol and/or SDS. However, it was unaffected by nuclease digestion or UV irradiation. Pruisner et al. identified a protein within this enriched infectious material which they termed PrP that was resistant to partial PK digestion and was found only in infected hamster brains. This protein migrated on SDS-PAGE at 27-30kDa and ultrastructurally had the appearance of rods, which were first identified by Merz et al. (Merz et al., 1981). Fractions enriched for these rods were found to be highly infectious. This PrP^{Sc} is thought to differ from the normal cellular PrP^{C} by conformation alone. FT-IR and CD studies have identified that PrP^{C} is about 40% α-helical with little ß-sheet, while PrP^{Sc} is ~30% α-helical and ~45% ß-sheet (Pan et al., 1993; Caughey et al., 1997; Safar et al., 1993). So far, no post-translational modifications have been

found that distinguish PrP^{Sc} from PrP^C despite numerous studies.

However, a remaining major problem is that even with current

techniques, the ratio of PrP^{Sc} molecules to infectious units is

on the order of 10,000 to one (Horwich and Weismann, 1997).

Hence, with such a ratio it is very difficult to completely rule

out that other essential components are not part of the

infectious agent or that some covalent or other post-

translational modifications do not occur as part of the PrP^C to

PrP^{Sc} conversion. Indeed, the pattern of glycosylation has been

shown to be distinctive among differing human PrP^{Sc} types,

indicating that this post-translational modification may

influence strain specificity (Collinge et al., 1996b). For the

final proof of the "protein only" hypothesis it will be necessary

to produce infectious particles *in vitro* from purified PrP^C or

from recombinant protein.

[0032]   With the purification of PrP27-30 it was possible to

obtain its amino terminal sequence and subsequently cDNA clones

encoding the PrP protein (Liao et al., 1986;Oesch et al., 1985).

The human PrP gene is found on chromosome 20. It spans 20 kb and

consists of a short, non-coding first exon, a 10-15 kb intron and

a second exon that contains the entire 759 bp open reading frame

and 1.64 kb of 3' non-translated sequence, with the translation

product being 253 amino acids long. The PrP gene is highly

conserved across mammalian species and is constitutively

expressed in both neuronal and non-neuronal tissue (Kretzschmar

et al., 1986). The highest mRNA levels are found in neurons, in

particular in the hippocampus; however, substantial amounts are

also found in the heart and skeletal muscle. The primary

structure of PrP from normal animals was found to be identical to

that found from scrapie infected animals and the levels of mRNA

were comparable in both settings (Oesch et al., 1985;Chesebro et

al., 1985). Cell culture studies have indicated that PrP^c is

transported in secretory vesicles to the external cell surface,

where it is anchored via a GPI moiety (Taraboulos et al., 1992).

Most of this PrP^c is internalized into an endocytic compartment;

however, some can be released into the extracellular space by

cleavage of the GPI anchor(Shyng et al., 1993). The endocytic

PrP^c is recycled intact to the cell surface or cleaved at the N-

terminus and externalized (Harris et al., 1993;Shyng et al.,

1993). The identification of the PrP gene, designated PRNP in

humans, also allowed for the characterization of numerous

mutations associated with familial prionoses (Prusiner et al.,

1998). Three mutations in the first putative α-helical domains

of PrP at codons 102, 105 and 117, with a fourth at codon 145,

which is at the carboxyl end of the second putative helical

domain, are associated with a GSS phenotype. The codon 145

mutation produces a stop codon and the synthesis of a truncated

PrP (Kitamoto et al., 1993). Other GSS linked mutations occur at

codons 198 and 217 (Dlouhy et al., 1992; Hsiao et al., 1992).

Interestingly, these two mutations are associated with widespread

NFT similar to that seen in AD. Conversely, a familial CJD

picture, with little or no amyloid deposition, has been

identified among kindreds with insertions of variable octarepeats

in the amino terminal domain of PrP and mutations at codons 178,

180, 200, 210 and 232; all these are in the third and fourth

putative helical domains of PrP (Prusiner et al., 1998). A

particularly interesting mutation occurs at codon 178, resulting

in a substitution of Asn for Asp. This point mutation can result

in either the clinical picture of CJD or FFI depending on a

polymorphism at codon 129 (Medori et al., 1992; Goldfarb et al.,

1992). The codon 178 mutation, plus Met at codon 129 results in

FFI whereas if there is a Val at codon 129 a CJD picture is seen.

This demonstrates the importance of the 129 codon polymorphism on

the distribution of disease, since in FFI the neuropathology is

largely confined to the thalamus while in CJD there is widespread

spongiform change in the cerebral cortex and in subcortical

nuclei.

[0033] Two critical genetic studies provide strong evidence

for the requirement of endogenous PrP^C for PrP^{Sc} infection. The

first of these used PrP knockout mice designated $Prnp^{0/0}$. In two

such lines of $Prnp^{0/0}$ mice, there were little or no differences

found from controls (Bueler et al., 1992; Manson et al., 1994);

however, altered sleep-wake cycles and abnormal synaptic behavior in brain slices have been reported (Tobler et al., 1996; Collinge et al., 1994). The synaptic changes could not be confirmed by one other group (Liedo et al., 1996). One line of Prnp$^{0/0}$ mice showed ataxia and Purkinje cell loss at about 70 weeks of age (Sakaguchi et al., 1996). Importantly, during transmission studies all of these different lines of Prnp$^{0/0}$ mice have all been shown to be highly resistant to prion infection (Bueler et al., 1993; Prusiner et al., 1993). Prnp$^{0/0}$ mice sacrificed at 4, 60, 120 and 315 days after inoculation with prions showed no infectivity with the exception of residual infectivity from the inoculum detected at five day following inoculation.

[0034]   The second set of genetic mouse experiments showing the need for endogenous PrP used mice expressing PrP with a GSS linked mutation. These mice spontaneously express prion disease which could be transferred when inoculated into transgenic mice expressing low levels of the same mutant PrP protein, but which otherwise would not develop disease (Telling et al., 1996; Hsiao et al., 1994). These transgenic mouse experiments combined with the Prnp$^{0/0}$ mice, clearly show the need for endogenous PrP^{C} for expression of disease.

[0035]   A ligand which interacts with PrP^{C} in the production of PrP^{Sc} has also been suggested and has been called "protein X" (Prusiner et al., 1998). The existence of protein X is indicated

by a number of transmission studies by Pruisner's team. They

found the transmission of human CJD prions to transgenic mice

expressing human PrP^c, Tg(HuPrP), occurred in only about 10% of

inoculated mice, which is similar to the rate seen in wild-type

mice (Telling et al., 1994). This situation was very different

when transgenic mice were used that express a chimeric

human/mouse (Hu/Mo) transgene (MH2M). The PrP^c encoded by MH2M

differed from Mo PrP^c by nine amino acids within residues 96 to

167, while there are 28 amino acid differences between the entire

mouse and human PrP sequences. All these mice, when inoculated

with human PrP^{Sc} become sick with fairly short incubation periods

of 202 to 249 days (Telling et al., 1995). The much higher

frequency of human CJD PrP^{Sc} transmission to Tg(MHu2M) mice

versus the Tg(HuPrP) shows the importance of the mouse PrP

sequence and may suggest that participation of a cellular factor

that recognizes some epitopes that are specific for mouse PrP

versus the human PrP. It is this cellular factor that has been

designated protein X.

[0036] Given our increasing knowledge of the factors involved

in the PrP^c to PrP^{Sc} conversion, its conceivable to design

effective therapeutic agents. Currently, no such therapeutic

agents exist. So far only a limited number of approaches have

been attempted. Experimental treatment approaches that have been

reported for prion diseases include the use of amphotericin B

(Pocchiari et al., 1987), Congo red (Caughey and Race, 1992),

sulphated polyanions (Ladogana et al., 1992), anthracyclines

(Tagliavini et al., 1997), β-sheet breaker peptides (Soto et al.,

2000), porphyrin and phthalocyanine compounds (Priola et al.,

2000). Some of these compounds delay the incubation time of

animals infected with PrP^{Sc} but all have limitations in terms of

toxicity and/or pharmacokinetics. Other approaches would be the

development of agents that either modify the action of protein X

or inhibit the conformational change of PrP^{C} to PrP^{Sc}.

Amylin

[0037]   Type-2 diabetes mellitus is characterized by defects in

the action of insulin and/or its secretion (Hoppener et al.,

2000). Usually both abnormalities are present but to a varying

degree, depending on the patient and the course of the disease.

Islet amyloid is found in about 90% of patients with type 2

diabetes, and there is a good correlation between the extent of

amyloid deposition and a reduction in insulin-producing β-cells,

suggesting that these deposits may lead to β-cell failure

(Howard, Jr., 1986). Identical deposits are found in other

species that develop this disease (Westermark et al., 1990), but

are absent in rodents (Westermark et al., 1990; Johnson et al.,

1989; Moriarty and Raleigh, 1999).

[0038]   The islet amyloid fibrils are composed of a 37 amino

acid peptide known as islet amyloid polypeptide or amylin

(Johnson et al., 1989). The nucleotide sequence of the amylin

gene is identical in normal subjects compared to diabetes

patients indicating that a change in amino acid sequence is not

the cause of amyloid fibril formation. An amyloidogenic region

(amino acids 20-29) has been identified in the human amylin

peptide by *in vitro* studies and by comparing the amino acid

sequences of various species in some of which amylin is not

deposited, such as rats, mice and hamsters (Westermark et al.,

1990; Johnson et al., 1989; Moriarty and Raleigh, 1999).

[0039]   Amylin fibrils are formed intracellularly and are also

found extracellularly. The mechanism of toxicity of these fibrils

has not been thoroughly investigated although integration of

aggregates into cell membranes, which can then function as

calcium permeable ion channels, has been implicated (Mirzabekov

et al., 1996). Subsequent increase in intracellular calcium may

lead to cytotoxcity. Disruption of intracellular membranes by

amyloid fibrils may also have a role in their toxicity (Janson et

al., 1999).

[0040]   Potential therapeutic targets for Type-2 diabetes

involve:

[0041]   A) Reducing the production of amylin or inhibiting

fibrillogenesis with: 1) drugs blocking amylin generating

enzymes; 2) drugs affecting the interaction between amylin and

its chaperones (Kisilevsky, 1996); 3) the use of antibodies

against amylin; and 4) compounds that bind to amylin and prevent

fibril formation and/or lead to fibril disassembly, an approach

used for other amyloid diseases (Soto et al., 1998; Sigurdsson et

al., 2000; Soto et al., 2000).

[0042]   B) Reducing the demand for endogenous insulin by

providing insulin therapy early in the course (Lindstrom et al.,

1997).

[0043]   C) Lowering glucose production (Zapecka-Dubno et al.,

1999).

[0044]   D) Increasing peripheral glucose disposal (Inzucchi et

al., 1998).

α-Synuclein

[0045]   Parkinson's disease (PD) is the second most common

neurodegenerative disease and the most common movement disorder

(Goedert, 2001). It affects 1-2% of the population over 65 years.

PD is primarily characterized by muscle rigidity, bradykinesia

and resting tremor. Lewy bodies, found to the greatest extent in

the substantia nigra, are the defining neuropathological

characteristics of all cases of PD and are mainly composed of α-

synuclein (Spillantini et al., 1997). The substantia nigra is

rich in dopaminergic neuronal cell bodies and is the main site of

cell loss in PD. Subsequent effects on the dopaminergic system are primarily responsible for the clinical symptoms. Cell loss is also seen in the motor nucleus of the vagus nerve, the hypothalamus, the nucleus basalis of Meynert, the locus coeruleus, the cerebral cortex, the olfactory bulb and the autonomic nervous system. Most of PD cases are idiopathic although a missense mutation in the α-synuclein gene is a rare genetic cause of PD (Polymeropoulos et al., 1997). Filamentous inclusions of this protein are also found in multiple system atrophy (MSA), such as olivopontocerebellar atropy, striatonigral degeneration, and Shy-Drager syndrome (Goedert, 2001). In MSA, glial cytoplasmic inclusions are observed instead of Lewy bodies. Another disease characterized by Lewy bodies and Lewy neurites is dementia with Lewy bodies (DLB), a common form of late onset dementia that often overlaps with Alzheimer's disease. Unlike PD, DLB has numerous Lewy bodies and Lewy neurites in the cerebral cortex (Kosaka, 1978), although the substantia nigra is also affected as in PD. Lewy bodies are also found in numerous rare diseases (Goedert, 2001).

[0046]   Synuclein proteins are abundant in the brain although their physiological function is not understood. The family consists of α-, β- and γ-synuclein ranging from 127 to 140 amino acids, which are 55-62% identical in amino acid sequences (Goedert, 2001). Of these three, only α-synuclein is associated

with diseases containing Lewy bodies and MSA with pathology

primarily in neurons and glia, respectively. Inactivation of the

α-synuclein gene does not lead to a severe neurological

phenotype, suggesting that loss of function of the protein does

[0047]   not lead to the neurodegeneration observed in PD

(Abeliovich et al., 2000).

[0048]   The accumulation of intracellular aggregates of α-

synuclein has been implicated in the pathogenesis of these

diseases although the mechanism of toxicity remains to be

determined. Assembly of α-synuclein is accompanied by the

conversion of α random coil formation to a β-pleated sheet

(Serpell et al., 2000; Conway et al., 2000; Biere et al., 2000),

and occurs through the repeats in the amino-terminal region,

whereas the carboxy-terminal regions inhibits assembly (Goedert,

2001). At least one of the α-synuclein mutations (A53T) leads to

generation of an altered α-synuclein with an increased rate of

filament formation. Although α-synuclein aggregates do not stain

with typical stains used to detect amyloid they have been shown

by electron diffraction to have the conformation characteristics

of amyloid fibrils (Serpell et al., 2000). β- and γ-synuclein do

not assemble into filaments (Serpell et al., 2000; Biere et al.,

2000; Giasson et al., 2001), which may explain why these forms

have not been implicated in any diseases.

[0049]   Although various treatment options are available for

PD, most of them affect the dopaminergic system or deal with the

side effects of those drugs (Hely et al., 2000). This is a

symptomatic treatment but does not address the cause of the

disease. Presently, there are no reports on experimental α-

synuclein based therapy.

Polyglutamine

[0050]   There are eight neurodegenerative disorders caused by

expansion of a CAG trinucleotide repeat coding for polyglutamine

(Kaytor and Warren, 1999). These diseases are Spinocerebellar

Ataxia Type 1 (39-83 glutamine repeats), Type 3 (55-84 repeats),

Type 6 (21-30 repeats), Type 7 (34 to over 200 repeats),

Huntington's disease (HD; 38-180 repeats), Spinal and Bulbar

Muscular Atrophy (38-65 repeats), and Dentatorubral-

Pallidoluysian Atropy (49-88 repeats). Protein aggregates have

been found in most of these diseases (Kaytor and Warren, 1999).

Their common features are progressive neuronal loss and decline

in motor and cognitive functions (Zoghbi and Orr, 2000). The

mechanism of pathogenesis has not been elucidated but the

polyglutamine repeats are important because longer repeats lead

to earlier onset and more severe phenotype (Kaytor and Warren,

1999; Hughes and Olson, 2001).

[0051]   Huntington's disease has been the most extensively investigated of these diseases. Nuclear inclusions of huntingtin are found both in neurons and in non-neuronal tissue outside the brain (Gutekunst et al., 1999). The striatum is predominantly affected in HD, although it contains far fewer protein aggregates than the cortex (Gutekunst et al., 1999), suggesting that some cell types are more vulnerable to the toxicity of the inclusions. There is a good correlation between the lengths of a truncated hutingtin protein and the size of the CAG repeat with the frequency and localization of aggregates (Rubinsztein et al., 1999). Huntingtin aggregation can be inhibited *in vitro* by antibodies and with compounds that bind to the β-sheet conformation of amyloid fibrils such as Congo red, thioflavin S, chrysamine G and Direct fast yellow (Heiser et al., 2000). Although HD is not considered an amyloid disease, these findings suggest that a similar therapy approach as is being developed for amyloid diseases may be effective for these diseases as well because the aggregates have a high β-sheet content. Other drug candidates include excitatory amino acid receptor antagonists, glutamate release inhibitors and mitochondrial agents (Hughes and Olson, 2001). Presently, no effective treatments have been developed for HD or the other polyglutamine diseases (Hughes and Olson, 2001).

[0052] In these diseases, the aggregates are found intra-
and/or extracellularily. Antibody mediated clearance of
extracellular Aβ deposits has already been observed by others and
us. With regard to the intracellular deposits, there are several
reports of cellular uptake of antibodies. It has been
demonstrated that a monoclonal antibody markedly inhibits rabies
virus RNA transcription following its cellular internalization
(Dietzschold et al., 1992). Also, motor neurons of the CNS in
rats that project outside the BBB seem capable of picking up IgG
from serum by retrograde transport (Fabian and Petroff, 1987).
Furthermore, extraction of IgG from the CSF by dendrites of
Purkinje cells has been demonstrated both in the rat (Borges et
al., 1985) and the guinea pig (Graus et al., 1991). In human
necropsy studies normal IgG has been detected in large amounts in
the cytoplasm of Purkinje cells (Fishman et al., 1990), and anti-
neuronal antibodies have been found in the human dorsal root
ganglia and Purkinje cells (Drlicek et al., 1992). Given these
reports, the antibodies may gain access into affected cells where
they may facilitate disassembly of the aggregates and/or prevent
their formation.

[0053] Citation of any document herein is not intended as an
admission that such document is pertinent prior art, or
considered material to the patentability of any claim of the
present application. Any statement as to content or a date of

any document is based on the information available to applicant

at the time of filing and does not constitute an admission as to

the correctness of such a statement.

SUMMARY OF THE INVENTION

[0054] The present invention provides an immunogenic but non-

deposit-forming polypeptide or peptide homologous to amyloid β,

to prion protein, to amylin, or α-synuclein, or to polyglutamine

repeat-containing proteins, which can be used for induction of an

immune response thereto and which would overcome or avoid the

complications and problems encountered in the prior art.

[0055] A synthetic immunogenic but non-amyloidogenic peptide

homologous to amyloid β according to the present invention

includes the first thirty amino acid residues of Aβ1-42 (SEQ ID

NO:1), where zero to five of residues 17-21 is substituted with

Lys, Asp, Glu, Pro, Gly, or Ser and preferably further includes

an N-terminal and/or C-terminal segment of 4-10 Lys or Asp

residues. Preferred peptides according to this embodiment

include, but are not limited to, SEQ ID NOS:2-5, 7-10, and 15-20.

[0056] A synthetic immunogenic but non-deposit-forming

polypeptide or peptide homologous to human (SEQ ID NO:21) or

bovine (SEQ ID NO:30) prion protein according to the present

invention includes either (1) the full-length prion protein where

one to five of residues 121, 122, 128, 129, and 130 of human

prion protein (PrP) or of residues 132, 133, 139, 140, and 141 of

bovine prion protein is substituted with Pro, Glu, Asp, Lys, Gly,

or Ser; (2) a fragment of the modified full-length prion protein

of (1) containing at least residues 90-144 of human prion protein

or residues 93-136 of bovine prion protein or; (3) a peptide

corresponding to residues 90-144 of human prion protein or to

residues 93-156 of bovine prion protein in which zero, or one to

five of residues 121, 122, 128, 129, 130 or 132, 133, 139, 140,

141, respectively, is substituted with Pro, Glu, Asp, Lys, Gly or

Ser and a polylysine or polyaspartate segment of 4 to 10 residues

is preferably present at the N-terminus and/or C-terminus.

Preferred peptides according to this embodiment include, but are

not limited to, SEQ ID NO:32 and SEQ ID NO:34-39, as well as SEQ

ID NO:33 and SEQ ID NO:40-45.

[0057] A synthetic immunogenic but non-deposit-forming peptide

homologous to human amylin according to the present invention

includes the sequence of human amylin (SEQ ID NO:46) where zero,

one, two, or three of residues 23, 26 and 27 is substituted with

Pro, Glu, Asp, Lys, Gly, or Ser and a polylysine or polyaspartate

segment of 4 to 10 residues is preferably present at the N-

terminus and/or C-terminus. Preferred peptides according to this

embodiment include, but are not limited to, SEQ ID NOS:48-53.

[0058] An immunogenic but non-deposit forming peptide

homologous to human α-synuclein according to the present

invention includes the full-length human α-synuclein (SEQ ID

NO:54) where one or more of the sets of valine residues, (1)

valine residues 37 and 40, (2) valine residues 48, 49, and 52,

and (3) residues 70, 71 and 74, is substituted with all Glu, all

Asp, all Pro, or all Lys residues, or includes a N-terminal

fragment of 30-36 residues in length or a C-terminal fragment of

30-66 residues in length of human α-synuclein either alone or

preferably joined at its N-terminus and/or C-terminus to a

polylysine or polyaspartate segment of 4-10 residues in length is

also provided by the present invention. Preferred peptides

according to this embodiment include, but are not limited to, SEQ

ID NO:55.

[0059]   A synthetic immunogenic, preferably non-deposit-

forming, polypeptide or peptide which contains a polyglutamine

segment of 30-200 glutamine residues in length joined at its N-

terminus and/or C-terminus to a polylysine or polyaspartate

segment of 4-10 residues in length.

[0060]   The present invention also provides a conjugate in

which a polypeptide or peptide of the present invention is cross-

linked to an immunostimulatory polymer molecule.

[0061]   Another aspect of the present invention is directed to

an immunizing composition/vaccine which contains an immunizing

effective amount of the immunogenic polypeptide or peptide of the

present invention, or a conjugate thereof.

[0062] The invention also provides a method of treating or preventing amyloid plaque forming diseases or amyloidosis by the use of a synthetic immunogenic but not deposit forming polypeptide or peptide homologous to the protein which forms the amyloid plaque. In one embodiment, the protein is homologous to the full length protein or peptide, in another embodiment, it includes only a portion of the protein or peptide which forms the amyloid plaque. In one embodiment, at least one residue is substituted with a different amino acid so as to decrease fibrillogenicity and in another embodiment it further includes a polylysine or polyaspartate segment (of 4-10) residues at the N-terminal and or the C-terminal.

[0063] In yet another embodiment, the invention provides a method of reducing amyloidosis comprising the step of administering an immunizing composition comprising a synthetic immunogenic but non-amyloidogenic peptide homologous to amyloid β, thereby reducing amyloidosis. In one embodiment, the synthetic peptide includes the first thirty amino acid residues of Aβ1-42 (SEQ ID NO:1), where zero to five of residues 17-21 are substituted with Lys, Asp, Glu, Pro, Gly, or Ser and preferably further includes an N-terminal and/or C-terminal segment of 4-10 Lys or Asp residues.

[0064] A further aspect of the present invention provides for a method for immunotherapy to induce an immune response to any

one of amyloid β peptides and amyloid deposits, prion protein and

prion deposits, amylin and amylin fibrils, α-synuclein and

deposits containing α-synuclein, or proteins with polyglutamine

repeats that are associated with neurodegenerative movement

disorders.

[0065]   A still further aspect of the invention provides for

molecules which include the antigen-binding portion of an

antibody specific for the immunogenic polypeptide or peptide of

the present invention, as well as for the preparation and use of

these molecules. Such molecules include, but are not limited to,

antibodies such as monoclonal antibodies, antibody fragments,

single chain antibodies, and humanized antibodies. Also provided

are pharmaceutical compositions containing such molecules or

antibodies together with one or more pharmaceutically acceptable

carriers, diluents, excipients or auxiliary agents, as well as

methods for reducing the formation of fibrils or deposits of

amyloid, prion, amylin, α-synuclein, or a protein with

polyglutamine repeats, by administering such compositions to a

subject, preferably a human subject, in need thereof.

[0066]   The invention also provides for the use of an

immunogenic but non-deposit-forming polypeptide or peptide

homologous to amyloid β, to prion protein, to amylin, or α-

synuclein, or to a polyglutamine repeat-containing protein, for

the preparation of a medicament for treating amyloidosis or a

disease or disorder characterized by deposits, fibrils, or aggregates of on or more of these polypeptides or peptides. In addition, the invention provides for molecules containing the antigen-binding portion of antibodies against an immunogenic but non-deposit-forming polypeptide or peptide of the invention, for use in the preparation of a medicament for treating a disease or disorder characterized by deposits, fibrils, or aggregates of one or more of these polypeptides or peptides. Such diseases and disorders include, but are not limited to, amyloidosis, Alzheimer's Disease, prionoses, type 2 diabetes or islet amyloidosis, Parkinson's Disease, and Huntington's Disease.

BRIEF DESCRIPTION OF THE DRAWINGS

[0067] Figure 1 shows the results of a thioflavin T fluorometric assay. Fibril formation of $A\beta 1$-42, $A\beta 1$-30-NH_2, and $K6A\beta 1$-30-NH_2 (SEQ ID NO:6) was measured *in vitro* following incubation at 37°C. $K6A\beta 1$-30-NH_2 was the only peptide that did not form fibrils at any of the time points.

[0068] Figures 2A and 2B show that $A\beta 40$ and $A\beta 42$ are toxic to human neuroblastoma cells (SK-N-SH) in culture as determined by the MTT assay, whereas $K6A\beta 30$-NH_2 has no effect at 2 days (Fig. 2A) and is slightly trophic at 6 days (Fig. 2B). *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$ compared to VEH group (one-way ANOVA).

[0069]   Figures 3A-3D show coronal sections (X50; original

magnification) stained with 6E10 against Aβ, through the

hippocampus and cortex in a Tg control-(Fig. 3A) and K6Aβ1-30-

treated (Fig. 3B) Tg mouse.   Figs. 3C and 3D are adjacent

sections (X100) double stained for interleukin-1 that recognizes

microglia, and Aβ. Note the reduction of amyloid burden in the

immunized mouse (Fig. 3B), and the lack of ramified microglia

(Fig. 3D) surrounding Aβ plaque in the same mouse, compared to a

control mouse (Fig. 3A, 3C). The bars in Figs. 3A and 3C are 100

μm. Abbreviations: hip = hippocampus; cx = cortex; cc = corpus

callosum.

[0070]   Figures 4A-4C show the reduction in cortical (Fig. 4A)

and hippocampal (Fig. 4B) amyloid burden (6E10) following 7

months treatment with K6Aβ1-30-NH$_2$. There is an 89% reduction in

cortical amyloid burden (*p = 0.0002; t-test; n = 4 per group)

and an 81% reduction in hippocampal amyloid burden (*p = 0.0001).

Soluble Aβ1-42 levels (Fig. 4C) are reduced by 57% within the

brains of the vaccinated mice (*p = 0.0019).

[0071]   Figure 5 shows the results of a thioflavin T

fluorometric assay. Fibril formation of Aβ1-42, Aβ1-40, Aβ1-30-

NH$_2$, Aβ1-30K6, Aβ1-30-NH$_2$(EE$_{18,19}$) and Aβ1-30-NH$_2$(DD$_{18,19}$) was

measured *in vitro* following incubation at 37°C for 15 days.

Within this period, no fibril formation of the Aβ derivatives

containing a polylysine segment or an amino acid substitution within the hydrophobic region was detected.

[0072] Figures 6A and 6B show the results of MTT cell toxicity assay. Neurotoxicity of $A\beta1-42$, $A\beta1-40$, $A\beta1-30-NH_2$, $K6A\beta1-30-NH_2$, $A\beta1-30K6$, $A\beta1-30-NH_2(EE_{18,19})$ and $A\beta1-30-NH_2(DD_{18,19})$ was determined following treatment of human neuroblastoma cells (SK-N-SH) for 2 (Fig. 6A) and 6 (Fig. 6B) days. $*p < 0.05$; $**p < 0.01$; $***p < 0.001$ compared to VEH group (one-way ANOVA). In this assay, $A\beta1-40$ and $A\beta1-42$ were toxic to human neuroblastoma cells (SK-N-SH) in culture. Of the $A\beta$ derivatives, even at the highest concentration (100 μM), only $A\beta1-30K6$ displayed a slight toxicity and only on day 2 of the test. Several of the peptides were neurotrophic following 6 days incubation. $*p<0.05$; $**p<0.01$; $***p<0.001$ (One-way Anova; Neuman Keuls' posthoc test).

[0073] Figure 7 shows the antibody titer determined by ELISA in mice 14 weeks after vaccination with mouse recPrP.

[0074] Figures 8A and 8B show that a higher anti-PrP^c (ME7 FAS PrP) antibody titer in vaccinated mice, as presented in Fig. 7, correlates with a longer incubation time in both PrP^{sc} inoculated mouse groups at lower dilution (Fig. 8A; $r^2=0.4389$, p=0.0052) and at higher dilution (Fig. 8B; $r^2=0.6786$, p<0.0001).

[0075] Figure 9 is a graph showing the effect of recPrP vaccination on disease onset, with day 0 being the first day an animal scored positive for disease. Group 1 mice were controls

inoculated with PrP^{Sc} at a 10 fold dilution, while group 2 was

inoculated at the same dilution but also received recPrP

vaccination. Group 3 mice were controls inoculated with PrP^{Sc} at

a 1000 fold dilution, while Group 4 received the same dilution of

PrP^{Sc} along with recPrP vaccination. The two control groups

received adjuvant and vehicle injections. Two way ANOVA shows a

significant effect for vaccination (p=0.0005) and PrP^{Sc} dilution

(p<0.000001). The Newman-Keuls post-hoc test showed vaccination

to have a stronger effect in the 10 fold dilution group (Group 1

versus 2, p=0.001 two-tailed; Group 3 versus 4, p=0.036 one-

tailed).

[0076] Figure 10 shows an alignment of amino acid sequences of

prion protein (PrP) from human (SEQ ID NO:21), gorilla (SEQ ID

NO:22), chimpanzee (SEQ ID NO:23), mouse (SEQ ID NO:24), rat (SEQ

ID NO:25), Syrian hamster (SEQ ID NO:26), mink (SEQ ID NO:27),

sheep (SEQ ID NO:28), goat (SEQ ID NO:29), cow (SEQ ID NO:30),

and greater kudu (SEQ ID NO:31). Amino acid residues that are

identical and conserved among the prion proteins of the species

presented in this figure are boxed.

[0077] Figures 11A-C show ELISA evaluation of sera from

individual animals vaccinated with K6Aβ1-30-NH2 and alum

adjuvant, testing for antibody titer against antigen (Fig. 11A),

Aβ1-42 (Fig. 11B) and Aβ1-40 (Fig. 11C).

[0078]   Figures 12A-C show ELISA evaluation of sera from

individual animals immunized with Aβ1-42 and alum adjuvant,

testing for antibody titer against antigen (Fig. 12A), K6Aβ1-30-

NH2 (Fig. 12B) and Aβ1-40 (Fig. 12C).

[0079]   Figures 13A and 13B depict a linear maze used to

evaluate cognitive capabilities of animals vaccinated with Aβ1-

30-NH$_2$ and K6Aβ1-30-NH$_2$ together with alum adjuvants, as well as

controls.   Fig. 13A shows the maze design during the adaptation

phase, and Fig. 13B during testing.   Dotted lines indicate

blocked alleys.

[0080]   Figures 14A-C depict results obtained from behavioral

studies of animals of about 3-4 months of age, after vaccination

with Aβ1-30-NH$_2$ and K6Aβ1-30-NH$_2$ together with alum adjuvants, as

well as controls.   The studies included testing of locomotor

activity (Fig. 14A), spontaneous avoidance (Fig. 14B), and

passive avoidance (Fig. 14C).   See Example 6.

[0081]   Figures 15A-N depict results obtained from behavioral

studies of animals of about 11 months of age, after vaccination

with Aβ1-30-NH$_2$ and K6Aβ1-30-NH$_2$ together with alum adjuvants, as

well as controls.   The studies included testing of locomotor

activity (Fig. 15A), and cognitive testing using traverse beam

(Figs. 15B and 15C), rotarod (Fig. 15D), radial arm maze (Figs.

15E and 15F), straight alley channel (Fig. 15G), visible platform

(Figs. 15H and 15I), Morris water maze (Figs. 15J and 15K), probe

trial (Figs. 15L and 15M), and linear maze (Fig. 15N). See Example 6.

DETAILED DESCRIPTION OF THE INVENTION

[0082] The present inventors have designed synthetic non-deposit-forming polypeptides/peptides homologous to amyloid β (Aβ), prion protein (PrP), amylin, α-synuclein, and polyglutamine repeat-containing proteins as an antigenic source. The peptide homologues have a reduced ability to adopt a β-sheet conformation, and have a lower risk of leading to any toxic effects in humans. By using these synthetic non-depositing-forming peptides, antibodies thereto, or conjugates thereof, in an immunizing composition, the present invention provides a means for rendering Aβ peptides and amyloid deposits, prion proteins and deposits, amylin and amylin fibrils, α-synuclein and deposits containing α-synuclein, or polyglutamine repeats and polyglutamine repeat-containing proteins as targets for the immune system. The amino acids in these peptides may be in either L- or D-form. D-form peptides can have a higher stability than L-form peptides *in vivo*.

[0083] An important feature provided by the present invention is a method for immunization which minimizes the toxicity associated with injected polypeptides/peptides, i.e., Aβ or other peptides, while maximizing the immune response to deposits and

polypeptides/peptides that make up the deposits, such as amyloid

deposits and Aβ peptides, prion deposits and prion protein, etc.

[0084] "Amyloidosis" as used herein refers to the deposition

of insoluble, fibrous amyloid (or "aggregate") proteins, which

are predominantly found extracellularly in organs and tissues.

Amyloid fibrils can consist of various amino acid sequences, but,

in general, all share β-pleated-sheet (or "β-sheet") secondary

structure. Amyloid proteins include, but are not limited to,

amyloid-β, amylin, prion protein, α-synuclein, and huntingtin.

See, also, Sigurdsson et al. (Trends Mol Med, 2002).

[0085] The term "reducing amyloidosis" refers herein after to

a decrease in either the amyloid plaques or deposits, number or

size, or both, or in another embodiment, a decrease in the

symptoms of the disease or another marker of the disease. Such

methods are known to those skilled in the art.

Amyloid β Variants

[0086] The synthetic non-amyloidogenic but immunogenic

peptides homologous to Aβ according to the present invention are

designed to have reduced fibrillogenic potential while

maintaining the two major immunogenic sites of Aβ peptides, which

are residues 1-11 and 22-28 of Aβ1-42 based on the antigenic

index of Jameson et al. (1988) and results/observations obtained

in the laboratory of the present inventors.

[0087]   In one embodiment, the non-amyloidogenic peptide comprises the first thirty amino acid residues (SEQ ID NO:1) of Ab1-42, wherein at least one of the hydrophobic residues at positions 17-21 of SEQ ID NO:1 are substituted with charged residues Lys, Asp, or Glu, or with residues Pro, Gly, or Ser.

[0088]   In another embodiment, the non-amyloidogenic peptide comprises the first thirty amino acid residues (SEQ ID NO:1) of Ab1-42, wherein one of the hydrophobic residues at positions 17-21 of SEQ ID NO:1 is substituted with charged residues Lys, Asp, or Glu, or with residues Pro, Gly, or Ser.

[0089]   In another embodiment, the non-amyloidogenic peptide comprises the first thirty amino acid residues (SEQ ID NO:1) of Ab1-42, wherein two of the hydrophobic residues at positions 17-21 of SEQ ID NO:1 are substituted with charged residues Lys, Asp, or Glu, or with residues Pro, Gly, or Ser.

[0090]   In another embodiment, the non-amyloidogenic peptide comprises the first thirty amino acid residues (SEQ ID NO:1) of Ab1-42, wherein three of the hydrophobic residues at positions 17-21 of SEQ ID NO:1 are substituted with charged residues Lys, Asp, or Glu, or with residues Pro, Gly, or Ser.

[0091]   In another embodiment, the non-amyloidogenic peptide comprises the first thirty amino acid residues (SEQ ID NO:1) of Ab1-42, wherein four of the hydrophobic residues at positions 17-

21 of SEQ ID NO:1 are substituted with charged residues Lys, Asp, or Glu, or with residues Pro, Gly, or Ser.

[0092] In another embodiment, the non-amyloidogenic peptide comprises the first thirty amino acid residues (SEQ ID NO:1) of Ab1-42, wherein five of the hydrophobic residues at positions 17-21 of SEQ ID NO:1 are substituted with charged residues Lys, Asp, or Glu, or with residues Pro, Gly, or Ser.

[0093] By modifying at least one residue at positions 17-21 of Ab1-30 (SEQ ID NO:1) with Lys, Asp, Glu, Pro, Gly, or Ser, which are residues that have a low probability of adopting β-sheet conformation, the fibrillogenic potential of the peptide is greatly reduced. SEQ ID NOs:12 and 13 are examples of such modified Aβ1-30. Furthermore, the presence of a series of Lys or Asp residues at the N-terminus and/or C-terminus of the synthetic peptide of the present invention further enhances immunogenicity (Werdelin, 1981) and reduce the propensity of the synthetic peptide to adopt a β-sheet conformation and form amyloid fibrils/deposits. The coupling of lysine residues to Aβ peptides of 4 to 8 residues in length has recently been proposed by Pallitto et al. (1999) in the design of anti-β-sheet peptides or Aβ fibrillogenesis inhibitors, but the use of Pallitto's peptides as immunogens has never been proposed. Polycationic amino acids have been previously used to enhance protein transport into cells by endocytosis/phagocytosis processes (Martinez-Fong et al.,

1994; Wang et al., 1989; Shen et al., 1985; Peterson et al.,

1984; Deierkauf et al., 1977; DiNicola et al., 2000). Buschle et

al., (1997) reported that polycationic amino acids enhanced

uptake of peptides by antigen presenting cells, thereby

initiating an immune response. They also reported that, whereas

peptide uptake mediated by polylysine appears to be due to an at

least transient permeabilization of cell membranes, peptide

delivery in the presence of polyarginine may rely on endocytic

processes.

[0094] The synthetic immunogenic but non-amyloidogenic peptide

homologous to Aβ according to the present invention, which is not

considered to be a peptide inhibitor of Aβ fibrillogenesis, is

represented by the formula
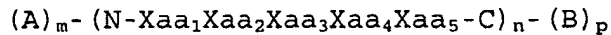
$$(A)_m-(N-Xaa_1Xaa_2Xaa_3Xaa_4Xaa_5-C)_n-(B)_p$$

wherein: m is 0, 4, 5, 6, 7, 8, 9, or 10;

           p is 0, 4, 5, 6, 7, 8, 9, or 10;

           A is Lys or Asp;

           B is Lys or Asp;

           n is 1 or 2;

           N is residues 1-16 of SEQ ID NO:1;

           C is residues 22-30 of SEQ ID NO:1;

           Xaa_1, Xaa_2, Xaa_3, Xaa_4, and Xaa_5 are Leu, Val, Phe, Phe,

and Ala, respectively, in which zero, one, two, three, four, or

five of residues Xaa$_1$, Xaa$_2$, Xaa$_3$, Xaa$_4$, and Xaa$_5$ is substituted

with Lys, Asp, Glu, Pro, Gly, or Ser; and

when zero residues are substituted, then either or both

of m or p is not zero.

[0095]   Some exemplary amino acid sequences of the peptide

represented by the above formula are presented and identified as

SEQ ID NOs:2-5 and 7-10.

[0096]   The basic thirty amino acid sequence (Aβ1-30) in which

zero or at least one of residues 17-21 are substituted is

represented in the above formula by N-Xaa$_1$Xaa$_2$Xaa$_3$Xaa$_4$Xaa$_5$-C.

This thirty amino acid residue segment can be repeated (n is 2)

in the synthetic peptide according to the present invention.

Preferably, a polylysine or polyaspartate segment of 4 to 10

residues is present at the N-terminus and/or the C-terminus of

the peptide.   When no residues are substituted in residues 17-21

of Aβ1-30, the peptide has a polylysine or polyaspartate segment

of 4 to 10 residues at the N-terminus and/or C-terminus.   If a

polylysine or polyaspartate segment is not present at the C-

terminus, then the C-terminus is preferably amidated, as

exemplified by SEQ ID NO:6 as a preferred embodiment.   SEQ ID

NO:11 is an embodiment of an unsubstituted Aβ1-30 peptide with a

polylysine or polyaspartate segment of 4 to 10 residues at the C-

terminus.

[0097]   Furthermore, when m is 0, the N-terminal polylysine or

polyaspartate segment of 4 to 10 residues is absent, and it is

then preferred that either the C-terminus of the peptide be

amidated to reduce the possibility that the C-terminal charge of

the peptide would reduce the immunogenicity of the residue 22-28

region of Aβ or that a polylysine or polyaspartate segment of 4

to 10 residue be present at the C-terminus. Another preferred

embodiment of the synthetic immunogenic but non-amyloidogenic

peptide according to the present invention is as follows:

when m is not zero, p is zero;

when p is not zero, m is zero; and

Xaa$_1$, Xaa$_2$, Xaa$_3$, Xaa$_4$, and Xaa$_5$ are Leu, Val, Phe,

Phe, and Ala, respectively, in which at least one residue of

Xaa$_1$, Xaa$_2$, Xaa$_3$, Xaa$_4$, and Xaa$_5$ is substituted with Pro, Asp,

Glu, Lys, Gly, or Ser (for example, SEQ ID Nos:2-5 and 7-10).

The invention provides for such synthetic

immunogenic but non-amyloidogenic peptides where all residues of

the peptide are L-amino acids or D-amino acids.

[0098]   In addition, the invention provides, in one embodiment,

a method of reducing amyloidosis comprising the step of

administering a composition comprising a synthetic immunogenic

but non-amyloidogenic peptide homologous to amyloid β, thereby

reducing amyloidosis. In one embodiment, the synthetic peptide

includes the first thirty amino acid residues of Aβ1-42 (SEQ ID

NO:1), where zero to five of residues 17-21 are substituted with
Lys, Asp, Glu, Pro, Gly, or Ser and preferably further includes
an N-terminal and/or C-terminal segment of 4-10 Lys or Asp
residues.

[0099]   In another embodiment, this invention provides a method
of treating or preventing amyloid plaque forming diseases or
amyloidoses by the use of a synthetic immunogenic but not deposit
forming polypeptide or peptide homologous to the protein which
forms the amyloid plaque. In one embodiment, the protein is
homologous to the full length protein or peptide, in another
embodiment, it includes only a portion of the protein or peptide
which forms the amyloid plaque. In one embodiment, at least one
residue is substituted with a different amino acid so as to
decrease fibrillogenicity and in another embodiment it further
includes a polylysine or polyaspartate segment (of 4-10) residues
at the N-terminal and or the C-terminal.

[00100] The invention provides, in one embodiment, a method of
reducing amyloidosis comprising the step of administering a
pharmaceutical composition comprising a synthetic immunogenic but
non-amyloidogenic peptide homologous to amyloid β, thereby
reducing amyloidosis. In one embodiment, the synthetic peptide
includes the first thirty amino acid residues of Aβ1-42 (SEQ ID
NO:1), where zero to five of residues 17-21 are substituted with
Lys, Asp, Glu, Pro, Gly, or Ser and preferably further includes

an N-terminal and/or C-terminal segment of 4-10 Lys or Asp

residues.

Prion

[00101] The synthetic immunogenic but non-deposit-forming

polypeptides or peptides homologous to human or bovine prion

protein (PrP) according to the present invention are designed

with considerations similar to the synthetic immunogenic but non-

amyloidogenic peptide homologous to Aβ according to the present

invention. However, one embodiment of the polypeptide or peptide

homologous to human or bovine prion protein is directed to a

full-length human or bovine prion protein in which one to five

residues, preferably four or five residues, of human prion

residues 121, 122, 128, 129, and 130, of SEQ ID NO:21 or of

bovine prion residues 132, 133, 139, 140, and 141 of SEQ ID NO:30

is substituted with Pro, Glu, Asp, Lys, Gly, or Ser, more

preferably Pro, Glu, Asp, or Lys. In addition, when more than

one residue is to be substituted, it is preferred that the same

amino acid residue is used for all substitutions.

[00102] It has been reported that the region of residues 90-144

of human PrP is important for initiating prion disease (Kanecko

et al., 2000), whereas residues 23-89 and 141-176 are not

required for infectivity. Accordingly, the embodiment of a full-

length prion protein with one to five amino acid substitutions

retains the epitopes located at approximately residues 93-119

145-174, and 172-201 that were previously reported to be

effective in raising antibodies. Any substitutions made in the

90-144 region reported to be important in initiating prion

disease in humans, which corresponds to the region of residues

93-156 in bovine PrP, is designed to replace residues that have a

high propensity for forming β-sheets, such as Val, Ile, Tyr, Trp,

Leu, Thr, Gln, and Met, according to Chou and Fasman with

residues that have a low propensity for forming β-sheets, such as

Pro, Glu, Asp, Lys, Gly, or Ser. The choice of residues 121,

122, 128, 129, and 130 of SEQ ID NO:21 and residues 132, 133,

139, 140, and 141 of SEQ ID NO:30 for substitution with residues

that have a low propensity for forming β-sheets (1) avoids

disturbing epitopes identified to be effective in raising

antibodies as well as the epitope at residues 132-140 of human

PrP to which the binding of an antibody prevents formation of the

abnormal scrapie form of prion protein (PrP^{Sc}) *in vitro* (Peretz

et al., 2001) and (2) results in a polypeptide that is

immunogenic but has a much reduced propensity for forming toxic

prion deposits.

[00103] An alternative to the full-length substituted/modified

human or bovine PrP for use as a synthetic immunogenic but non-

deposit forming polypeptide or peptide homologous to human or

bovine PrP, is a segment of the sequence of the full-length

unmodified or substituted/modified human (SEQ ID NO:32) or bovine (SEQ ID NO:33) PrP, containing at least residues 90 to 144 of SEQ ID NO:32 or at least residues 93 to 156 of SEQ ID NO:33, alone or joined at its N-terminus and/or C-terminus to a polyaspartate or a polylysine of 4 to 10 residues in length.

[00104] Additional preferred embodiments of the synthetic immunogenic but non-deposit-forming peptide homologous to human or bovine PrP include peptides of residues 90 to 144 of SEQ ID NO:21 or of residues 93 to 156 of SEQ ID NO:30 or fragments of the peptides, where one to five residues but preferably four or five residues are substituted, and/or a polylysine or polyaspartate of 4 to 10 residues in length is joined at the N-terminal and/or C-terminal end of the peptide. These additional preferred embodiments are represented by the formula
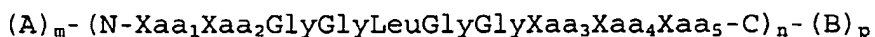
$(A)_m$-$(N$-$Xaa_1Xaa_2GlyGlyLeuGlyGlyXaa_3Xaa_4Xaa_5$-$C)_n$-$(B)_p$

wherein: m is 0, 4, 5, 6, 7, 8, 9, or 10;

p is 0, 4, 5, 6, 7, 8, 9, or 10;

A is Lys or Asp;

B is Lys or Asp;

n is 1 or 2;

N represents residues 90-120 of SEQ ID NO:21;

C represents residues 131-144 of SEQ ID NO:21;

Xaa_1, Xaa_2, Xaa_3, Xaa_4, and Xaa_5 are Val, Val, Tyr, Met, and Leu, respectively, in which zero or one to five, preferably

four or five, of residues Xaa_1, Xaa_2, Xaa_3, Xaa_4, and Xaa_5 is

substituted with Pro, Glu, Asp, Lys, Gly, or Ser; and

[00105] when zero residue is substituted, then either or both m

and p is not zero (SEQ ID NOs: 34-39).

[00106] Where the peptide homologous to bovine PrP is used for

administration in cows, N represents residues 93-131 of SEQ ID

NO:30 and C represents residues 142-156 of SEQ ID NO:30 and the

peptides have the sequences of SEQ ID NOs:40-45. The presence or

absence of polylysine or polyaspartate at the N-terminus and/or

C-terminus thereof or the presence or absence of amidation at the

C-terminus is as discussed above for the synthetic immunogenic

but non-amyloidogenic peptide homologous to Aβ.

[00107] Another embodiment of the synthetic immunogenic but

non-deposit-forming polypeptide/peptide homologous to human or

bovine PrP is where all residues of the polypeptide/peptide are

D-amino acid residues.

Amylin

[00108] The synthetic but non-deposit-forming peptide

homologous to human amylin is also designed with considerations

similar to the synthetic but non-amyloidogenic peptide homologous

to Aβ according to the present invention. Human amylin is a 37

amino acid peptide having the amino acid sequence of SEQ ID NO:46

and containing an amyloidogenic region between residues 20 to 29

of SEQ ID NO:46. Mouse amylin is also 37 residues in length but having the amino acid sequence of SEQ ID NO:47, which differs from human amylin at residues 18, 23, 25, 26, 28, and 29. Unlike human amylin, mouse amylin does not form fibril, most likely because three of the residues within the region of residues 20 to 29 differs from human amylin in that they are proline residues.

[00109] The same approach that is applied to the peptides homologous to Aβ or prion protein is applied here with respect to human amylin residues 23, 26, and 27 of SEQ ID NO:46, where any one or more of these three residues having a high propensity for forming β-sheets according to Chou and Fasman is substituted with Pro, Glu, Asp, Lys, Gly, or Ser residues which have a low propensity of forming β-sheets. The preferred embodiments of the synthetic immunogenic but non-amyloidogenic (non-deposit-forming) peptide homologous to human amylin according to the present invention are represented by the formula
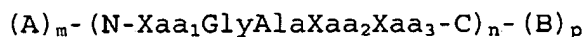
$$(A)_m\text{-}(N\text{-}Xaa_1GlyAlaXaa_2Xaa_3\text{-}C)_n\text{-}(B)_p$$

wherein: m is 0, 4, 5, 6, 7, 8, 9, or 10;

p is 0, 4, 5, 6, 7, 8, 9, or 10;

A is Lys or Asp;

B is Lys or Asp;

n is 1 or 2;

N represents residues 1-22 of SEQ ID NO:46;

C represents residues 28-37 of SEQ ID NO:46;

Xaa$_1$, Xaa$_2$, and Xaa$_3$ are Phe, Ile, and Leu,

respectively, in which zero, one, two or three of residues Xaa$_1$,

Xaa$_2$, and Xaa$_3$ is substituted with Pro, Asp, Glu, Lys, Gly, or

Ser; and

when zero residues is substituted, then either or both

m and p is not zero (SEQ ID NOs:48-53).

[00110] Similar to the synthetic immunogenic

polypeptides/peptides homologous to Aβ or PrP according to the

present invention as discussed above, another embodiment of the

synthetic immunogenic peptide homologous to human amylin is where

all residues are D-amino acids.

α-Synuclein

[00111] Human α-synuclein is 140 amino acid residues in length

(SEQ ID NO:54). The N-terminus contains imperfect 11-amino-acid

repeats with the consensus sequence KTKEGV (corresponding to

residues 32-37 of SEQ ID NO:54). Following the repeats is a

hydrophobic intermediate region and a negatively charged C-

terminus. Full-length α-synuclein is found in the filaments of

Lewy bodies, its C-terminal region being exposed, whereas the

amino-terminal region is buried and exposed only at one end.

Assembly of α-synuclein occurs through the repeats in the amino-

terminus, whereas the carboxy-terminal region inhibits assembly.

The transformation of soluble α-synuclein to its fibrillar

disease-associated form requires a conformational change (increased β-sheet content).

[00112] The synthetic immunogenic but non-deposit-forming polypeptide or peptide homologous to human α-synuclein according to the present invention is a polypeptide of SEQ ID NO:55 in which one or more of three sets of valine residues, (1) residues 37 and 40, (2) residues 48, 49, and 52, and (3) residues 70, 71, and 74 is substituted with all Glu, all Asp, all Pro, all Lys, all Gly, or all Ser residues. The substitutions makes the polypeptide still immunogenic but with a low propensity to form toxic protofibrils and/or aggregates.

[00113] Alternatively, shorter synthetic peptides which consist of either (1) the first N-terminal 30 to 36 residues of the human α-synuclein of SEQ ID NO:54, alone or joined at its N-terminal and/or C-terminal to a polylysine or polyaspartate segment of 4 to 10 residues in length or (2) the last C-terminal 30 to 66 residues of the human α-synuclein of SEQ ID NO:54, alone or joined at its N-terminus and/or C-terminus to a polylysine or polyaspartate segment of 4 to 10 residues in length can be used as the synthetic immunogenic peptide of the present invention. As an additional embodiment, the residues of the full-length modified α-synuclein or the shorter synthetic peptides of the present invention may all be D-amino acids.

[00114] The term "Lewy bodies" as used herein refers to intracytoplasmic, eosinophilic, round to elongated inclusions mainly composed of α-synuclein. Lewy bodies can be found in vacuols of injured or fragmented neurons, and are indicative of Parkinson's Disease (PD), variant of Alzheimer's disease with Lewy bodies, and dementia with Lewy bodies (DLB).

Polyglutamine

[00115] According to the present invention, the synthetic immunogenic but non-deposit-forming polypeptide or peptide homologous to polyglutamine repeats in proteins that are associated with neurodegenerative movement disorders has a polyglutamine segment of 30-200 glutamine residues in length joined at its N-terminus and/or C-terminus to a polylysine or polyaspartate segment of 4-10 residues in length.

[00116] This aspect of the present invention also encompasses a method for inducing an immune response to a protein with polyglutamine repeats that is associated with neurodegenerative movement disorders, where the method involves administering to a human subject in need thereof an immunizing composition containing a polyglutamine repeat-containing polypeptide or peptide of the present invention.

Peptide Design

[00117] Those of skill in the art will also appreciate that peptidomimetics of the synthetic immunogenic but non-deposit-forming polypeptide or peptide of the present invention, where the peptide bonds are replaced with non-peptide bonds, can also be used. Peptidomimetics can have various different structures (Ripka et al., 1998). For example, peptidomimetics can be: (1) peptide analogues containing one or more amide bond replacements (Spatola, 1983); (2) peptide analogues with various conformational restrains (Hart and Rich 1996), (3) novel structures that replace the entire peptide backbone while retaining isosteric topography of the peptide (Farmer, 1980), and (4) various heterocyclic natural products or screening leads that mimick the function of the natural peptide (Fletcher and Campell, 1998). Any suitable peptidomimetic can be used in the context of the present invention.

[00118] Antibodies to peptides wherein the amino acids are in D-form (i.e., D-amino acids) recognize also the corresponding L-form peptide, and vice versa (Benkirane et al., 1993). Accordingly, in one embodiment of the synthetic immunogenic but non-amyloidogenic peptide according to the present invention, all residues of the peptide are D-amino acids. The amino acids being in D-form would also have the effect of enhancing the stability of the peptide. These D-amino acids can be in the same order as

the L-form of the peptide or assembled in a reverse order from

the L-form sequence to maintain the overall topology of the

native sequence (Ben-Yedidia et al., 2002).

[00119] The reduced fibrillogenic or reduced deposit-forming

potential for the synthetic polypeptide or peptide according to

the present invention can be readily determined by measuring the

β-sheet conformation of the polypeptides/peptides using

conventional techniques such as circular dichroism spectra, FT-

IR, and electron microscopy of polypeptide or peptide

suspensions.

[00120] It is also well-known that immunogens must be presented

in conjunction with major histocompatibility (MHC) class II

antigens to evoke an efficient antibody response. The MHC class

II antigens produced by antigen-presenting cells (APCs) bind to T

cell epitopes present in the immunogen in a sequence specific

manner. This MHC class II-immunogen complex is recognized by

$CD4^+$ lymphocytes (T_h cells), which cause the proliferation of

specific B cells capable of recognizing a B cell epitope from the

presented immunogen and the production of B cell epitope-specific

antibodies by such B cells.

[00121] Accordingly, in one embodiment, the immunogenicity of

the synthetic peptides of the present invention can be increased

by forming a conjugate with an immunostimulatory polymer molecule

such as mannan (polymer of mannose), glucan (polymer of β1-2

glucose), tripalmitoyl-S-glycerine cysteine, and peptides which

are currently approved for use in vaccines in humans. Such

peptides, approved for use in vaccines, provide strong T helper

cell (T_h) epitopes from potent immunogens such as tetanus toxin,

pertussis toxin, the measles virus F protein, and the hepatitis B

virus surface antigen (HBsAg). The T_h epitopes selected to be

conjugated to the synthetic peptide are preferably capable of

eliciting T helper cell responses in large numbers of individuals

expressing diverse MHC haplotypes. These epitopes function in

many different individuals of a heterogeneous population and are

considered to be promiscuous T_h epitopes. Promiscuous T_h

epitopes provide an advantage of eliciting potent antibody

responses in most members of genetically diverse population

groups.

[00122] Moreover, the T helper cell epitopes conjugated/cross-

linked to the synthetic peptide of the present invention are also

advantageously selected not only for a capacity to cause immune

responses in most members of a given population, but also for a

capacity to cause memory/recall responses. When the mammal is

human, the vast majority of human subjects/patients receiving

immunotherapy with the synthetic peptide of the present invention

will most likely already have been immunized with the pediatric

vaccines (i.e., measles+mumps+rubella and diphtheria+pertussis+

tetanus vaccines) and, possibly, the hepatitis B virus vaccine.

These patients have therefore been previously exposed to at least one of the T_h epitopes present in pediatric vaccines. Prior exposure to a T_h epitope through immunization with the standard vaccines should establish T_h cell clones which can immediately proliferate upon administration of the synthetic peptide (i.e., a recall response), thereby stimulating rapid B cell responses to $A\beta$ peptides and amyloid deposits.

[00123] While the T_h epitopes that may be used in the conjugate with the synthetic peptide of the invention are promiscuous, they are not universal. This characteristic means that the T_h epitopes are reactive in a large segment of an outbred population expressing different MHC antigens (reactive in 50 to 90% of the population), but not in all members of that population. To provide a comprehensive, approaching universal, immune reactivity for the synthetic non-deposit-forming peptide according to the present invention, a mixture of conjugates with different T_h epitopes cross-linked to a synthetic peptide can be prepared. For example, a combination of four conjugates with promiscuous T_h epitopes from tetanus and pertussis toxins, measles virus F protein and HBsAg may be more effective.

[00124] The T_h epitopes in the immunostimulatory peptide cross-linked to the synthetic non-deposit-forming peptide according to the present invention include hepatitis B surface antigen T helper cell epitopes, pertussis toxin T helper cell epitopes,

tetanus toxin T helper cell epitopes, measles virus F protein T

helper cell epitope, *Chlamydia trachomitis* major outer membrane

protein T helper cell epitopes, diphtheria toxin T helper cell

epitopes, *Plasmodium falciparum* circumsporozoite T helper cell

epitopes, *Schistosoma mansoni* triose phosphate isomerase T helper

cell epitopes, *Escherichia coli* TraT T helper cell epitopes and

are disclosed in U.S. Patent 5,843,446, the entire disclosure of

which is incorporated herein by reference.

[00125] Peptide Orthologues. In a particular embodiment, the

invention provides immunizing compositions based on orthologues

to amyloid proteins. Because it is expected that mammalian

species such mouse, rat, sheep, goat, mink, Syrian hamster, and

greater Kudu (an antelope) do not transmit prion disease to

humans, an immunizing composition with a prion protein or an

immunogenic fragment thereof from such a mammalian species, like

an immunizing composition with a synthetic immunogenic but non-

deposit forming polypeptide or peptide homologous to human PrP,

can be administered to a human subject in need thereof to induce

an immune response to prion protein and prion deposits. From the

amino acid alignment shown in Fig. 10, it is further expected

that a prion protein, where the conserved amino acid residues

that correspond to those amino acids substituted in the modified

human PrP of SEQ ID NO:32 are likewise substituted, can also be

administered to a human subject to induce an immune response to

prion protein and prion deposits. For instance, conserved residues 120, 121, 127, 128, and 129 of mouse PrP correspond to residues 121, 122, 128, 129, and 130 of human PrP and can be likewise substituted.

[00126] Similarly, in a method for inducing an immune response to PrP and prion deposit in a bovine subject, an immunizing composition with a synthetic immunogenic but non-deposit-forming polypeptide/peptide homologous to bovine PrP according to the present invention or an immunizing composition with a prion protein, or immunogenic fragment thereof, from a mammalian species that does not transmit prion disease to cows can be administered. The prion protein or fragment thereof from a mammalian species that does not transmit prior disease to cows may be modified at either or both termini or at the corresponding conserved amino acid residues according to the synthetic immunogenic but non-depositing forming polypeptide/peptide homologous to bovine PrP.

[00127] In a method for inducing an immune response to amylin and amylin fibrils, an immunizing composition with a synthetic immunogenic but non-deposit-forming peptide homologous to human amylin according to the present invention or an immunizing composition with an amylin from a mammalian species that does not form amylin fibrils, such as rodents, is administered to a human subject in need thereof.

[00128] With regard to the method for inducing an immune response to α-synuclein and deposits containing α-synuclein such as Lewy bodies, the synthetic immunogenic but non-deposit-forming polypeptide/peptide homologous to human α-synuclein or an α-synuclein from a mammalian species which does not form Lewy bodies, such as rodents (mouse, rat, hamster) is administered in an immunizing composition to a human subject in need thereof.

Peptide Preparation

[00129] It will be appreciated by those of skill in the art that the term "synthetic" as used with the peptide of the present invention means that it is either chemically synthesized or is produced in an organism only when the host organism is genetically transformed from its native state to produce the peptide. The synthetic peptides of the present invention can be made by synthetic chemical methods which are well known to the ordinary skilled artisan. Accordingly, the synthetic peptides can be synthesized using the automated Merrifield techniques of solid phase synthesis with either t-Boc or F-moc chemistry on Peptide Synthesizers such as an Applied Biosystems Peptide Synthesizer.

[00130] Alternatively, polypeptides or longer peptides can be synthesized by well-known recombinant DNA techniques. Any standard manual on DNA technology provides detailed protocols to

produce the synthetic polypeptides/peptides of the invention. To

construct a nucleotide sequence encoding a synthetic

polypeptide/peptide of the present invention, the amino acid

sequence is converted into an encoding nucleic acid sequence, and

preferably using optimized codon usage for the organism in which

the polypeptide/peptide will be expressed. Next, a synthetic

gene is made, typically by synthesizing overlapping

oligonucleotides which encode the peptide and any regulatory

elements, if necessary. The synthetic gene is inserted in a

suitable cloning vector and recombinant clones are obtained and

characterized. The synthetic polypeptide/peptide of the present

invention is then expressed under suitable conditions appropriate

for the selected expression system and host, and the desired

polypeptide/peptide is purified and characterized by standard

methods.

[00131] An immunostimulatory peptide that can be cross-linked

to the synthetic non-deposit-forming peptide of the invention is

also obtainable from the invasin protein of a *Yersinia* species.

The invasins of the pathogenic bacteria *Yersinia spp.* are outer

membrane proteins which mediate entry of the bacteria into

mammalian cells (Isberg et al., 1990). Invasion of cultured

mammalian cells by the bacterium was demonstrated to require

interaction between the *Yersinia* invasin molecule and several

species of the $\beta 1$ family of integrins present on the cultured

cells (Tran Van Nhieu et al., 1991) Since T lymphocytes are rich

in β1 integrins (especially activated immune or memory T cells)

the effects of invasin on human T cell have been investigated

(Brett et al., 1993). It is thought that integrins facilitate

the migration of immune T cells out of the blood vessels and

through connective tissues to sites of antigenic challenge

through their interaction with extracellular matrix proteins

including fibronectin, laminin and collagen. The carboxy-

terminus of the invasin molecule was found to be co-stimulatory

for naive human CD4^{+} T in the presence of the non-specific

mitogen, anti-CD3 antibody, causing marked proliferation and

expression of cytokines. The specific invasin domain which

interacts with the β1 integrins to cause this stimulation also

was identified (Brett et al., 1993). Because of the demonstrated

T cell co-stimulatory properties associated with this domain, it

can be cross-linked to the synthetic peptide of the present

invention to enhance immunogenicity.

[00132] Many of the outer membrane proteins of Gram-negative

bacteria are both lipid-modified and very immunogenic. Because

of the apparent correlation between covalent lipid linkage and

immunogenicity, tripalmitoyl-S-glycerine cysteine (Pam$_3$Cys), a

lipid common to bacterial membrane proteins, can be coupled to

the synthetic polypeptides/peptides in a conjugate to also

enhance immunogenicity.

Adjuvants

[00133] Immunogenicity can further be significantly improved if
the synthetic polypeptides/peptides are co-administered with
adjuvants. Adjuvants enhance the immunogenicity of an antigen
but are not necessarily immunogenic themselves. Adjuvants may
act by retaining the antigen locally near the site of
administration to produce a depot effect facilitating a slow,
sustained release of antigen to cells of the immune system.
Adjuvants can also attract cells of the immune system to an
antigen depot and stimulate such cells to elicit immune
responses.

[00134] Immunostimulatory agents or adjuvants have been used
for many years to improve the host immune responses, e.g. to
vaccines. Intrinsic adjuvants, such as lipopolysaccharides,
normally are the components of the killed or attenuated bacteria
used as vaccines. Extrinsic adjuvants are immunomodulators which
are typically non-covalently linked to antigens and are
formulated to enhance the host immune responses. Thus, adjuvants
have been identified that enhance the immune response to antigens
delivered parenterally. Some of these adjuvants are toxic,
however, and can cause undesirable side-effects, making them
unsuitable for use in humans and many animals. Indeed, only
aluminum hydroxide and aluminum phosphate (collectively commonly

referred to as alum) are routinely used as adjuvants in human and

veterinary vaccines. The efficacy of alum in increasing antibody

responses to diphtheria and tetanus toxoids is well established

and a HBsAg vaccine has been adjuvanted with alum as well.

Particularly suitable amuminum-based adjuvants include

Alhydrogel® and Adju-Phos® (both from Superfos Biosector,

Denmark).

[00135] A wide range of extrinsic adjuvants can provoke potent

immune responses to antigens. These include saponins complexed

to membrane protein antigens (immune stimulating complexes),

pluronic polymers with mineral oil, killed mycobacteria in

mineral oil, Freund's complete adjuvant, bacterial products, such

as muramyl dipeptide (MDP) and lipopolysaccharide (LPS), as well

as lipid A, and liposomes. To efficiently induce humoral immune

responses (HIR) and cell-mediated immunity (CMI), immunogens are

emulsified in adjuvants. Many adjuvants are toxic, inducing

granulomas, acute and chronic inflammations (Freund's complete

adjuvant, FCA), cytolysis (saponins and Pluronic polymers) and

pyrogenicity, arthritis and anterior uveitis (LPS and MDP).

Although FCA is an excellent adjuvant and widely used in

research, it is not licensed for use in human or veterinary

vaccines because of its toxicity.

[00136] U.S. Pat. No. 4,855,283 teaches glycolipid analogues

including N-glycosylamides, N-glycosylureas and N-

glycosylcarbamates, each of which is substituted in the sugar

residue by an amino acid, as immuno-modulators or adjuvants.

U.S. Pat. No. 4,258,029 teaches that octadecyl tyrosine

hydrochloride (OTH) functions as an adjuvant when complexed with

tetanus toxoid and formalin inactivated type I, II and III

poliomyelitis virus vaccine. Also, Nixon-George et al., 1990,

reported that octadecyl esters of aromatic amino acids complexed

with a recombinant hepatitis B surface antigen enhanced the host

immune responses against hepatitis B virus.

[00137] The addition of exogenous adjuvant/emulsion

formulations which maximize immune responses to the synthetic

non-deposit-forming polypeptides/peptide are preferred. The

adjuvants and carriers that are suitable are those: (1) which

have been successfully used in Phase I human trials; (2) based

upon their lack of reactogenicity in preclinical safety studies,

have potential for approval for use in humans; or (3) have been

approved for use in food and companion animals. Some of the

adjuvants that are currently undergoing clinical tests are

reported in Aguado et al., (1999).

Formulation and Administration

[00138] Immunotherapy regimens which produce maximal immune

responses following the administration of the fewest number of

doses, ideally only one dose, are highly desirable. This result

can be approached through entrapment of immunogen in
microparticles. For example, the absorbable suture material
poly(lactide-co-glycolide) co-polymer can be fashioned into
microparticles containing immunogen. Following oral or
parenteral administration, microparticle hydrolysis *in vivo*
produces the non-toxic byproducts, lactic and glycolic acids, and
releases immunogen largely unaltered by the entrapment process.
The rate of microparticle degradation and the release of
entrapped immunogen can be controlled by several parameters,
which include (1) the ratio of polymers used in particle
formation (particles with higher co-glycolide concentrations
degrade more rapidly); (2) particle size, (smaller particles
degrade more rapidly than larger ones); and, (3) entrapment
efficiency, (particles with higher concentrations of entrapped
antigen degrade more rapidly than particle with lower loads).
Microparticle formulations can also provide primary and
subsequent booster immunizations in a single administration by
mixing immunogen entrapped microparticles with different release
rates. Single dose formulations capable of releasing antigen
ranging from less than one week to greater than six months can be
readily achieved. Moreover, delivery of the synthetic
polypeptide/peptide according to the present invention entrapped
in microparticles can also provide improved efficacy when the

microparticulate immunogen is mixed with an exogenous

adjuvant/emulsion formulations.

[00139] The efficacy of the synthetic polypeptides/peptides can

be established and analyzed by injecting an animal, e.g., mice or

rats, with the synthetic polypeptide/peptide formulated in alum

and then following the immune response to, e.g., amyloid β

peptides, prion protein, amylin, α-synuclein, or polyglutamine,

as described below.

[00140] Another aspect of the present invention provides an

immunizing composition which includes an immunizing effective

amount of one or more of the synthetic polypeptides/peptides of

the invention, or conjugates thereof, and a pharmaceutically

acceptable carrier, excipient, diluent, or auxiliary agent,

including adjuvants. Accordingly, the synthetic

polypeptides/peptides, or conjugates thereof, can be formulated

as an immunizing composition using adjuvants, pharmaceutically-

acceptable carriers, excipients, diluents, auxiliary agents or

other ingredients routinely provided in immunizing compositions.

Such formulations are readily determined by one of ordinary skill

in the art and include formulations for immediate release and for

sustained release, e.g., microencapsulation. The present

immunizing compositions can be administered by any convenient

route including subcutaneous, oral, intramuscular, or other

parenteral or internal route. Similarly the vaccines can be

administered as a single dose or divided into multiple doses for

administration. Immunization schedules are readily determined by

the ordinary skilled artisan. For example, the adjuvants or

emulsifiers that can be used in this invention include alum,

incomplete Freund's adjuvant, liposyn, saponin, squalene, L121,

emulsigen and ISA720. In preferred embodiments, the

adjuvants/emulsifiers are alum, incomplete Freund's adjuvant, a

combination of liposyn and saponin, a combination of squalene and

L121 or a combination of emulsigen and saponin.

[00141] The immunizing compositions of the present invention

contain an immunoeffective amount of one or more of the synthetic

polypeptides/peptides or conjugates thereof and a

pharmaceutically acceptable carrier. Such compositions in dosage

unit form can contain about 0.5 µg to about 1 mg of each peptide

or conjugate per kg body weight. When delivered in multiple

doses, the dosage unit form is conveniently divided into the

appropriate amounts per dosage.

[00142] Immunizing compositions which contain cocktails of two

or more of the synthetic polypeptides/peptides, or conjugates

thereof, of the present invention enhance immunoefficacy in a

broader population and thus provide a better immune response to

amyloid β peptides and amyloid fibrils, to prion proteins and

prion deposits, to amylin and amylin deposits, or to α-synuclein

and deposits containing α-synuclein. Other immunostimulatory

synthetic polypeptide/peptide immunogens are arrived at through

modification into lipopeptides so as to provide built-in

adjuvanticity for potent vaccines. The immune response to

synthetic polypeptide/peptide immunogens of the present invention

can be improved by delivery through entrapment in or on

biodegradable microparticles of the type described by O'Hagan et

al (1991). The immunogens can be encapsulated with or without

adjuvant, including covalently attached lipid moiety such as

Pam_3Cys, and such microparticles can be administered with an

immunostimulatory adjuvant such as Freund's Incomplete Adjuvant

or alum. The microparticles function to potentiate immune

responses to an immunogen and to provide time-controlled release

for sustained or periodic responses, for oral administration, and

for topical administration (O'Hagan et al., 1991).

[00143] A further aspect of the present invention is a method

for immunization with the synthetic polypeptide/peptide or

conjugate thereof of the present invention. This method

according to the present invention involves administering to a

mammal in need thereof, preferably human, an immunizing

composition containing the synthetic polypeptide(s)/peptide(s) or

conjugates thereof. With respect to induction of an immune

response to amyloid β and amyloid β deposits, efficacy will be

tested first in transgenic mouse models of AD such as the mouse

model used in Schenk et al. (1999) or other publicly or

commercially available AD transgenic mouse model.

Anti-Peptide Antibodies

[00144] Yet another aspect of the present invention provides

for antibodies raised against the immunogenic polypeptides/

peptides of the present invention and molecules which includes

the antigen-binding portion of such antibodies.

[00145] It should be understood that when the term "antibodies"

is used with respect to the antibody embodiments of the present

invention, this is intended to include intact antibodies, such as

polyclonal antibodies or monoclonal antibodies (mAbs), as well as

proteolytic fragments thereof such as the Fab or F(ab')$_2$

fragments. Furthermore, the DNA encoding the variable region of

the antibody can be inserted into other antibodies to produce

chimeric antibodies (see, for example, U.S. Patent 4,816,567) or

into T-cell receptors to produce T-cells with the same broad

specificity (see Eshhar, *et al.*, (1990) and Gross *et al.*,

(1989)). Single chain antibodies can also be produced and used.

Single chain antibodies can be single chain composite

polypeptides having antigen binding capabilities and comprising a

pair of amino acid sequences homologous or analogous to the

variable regions of an immunoglobulin light and heavy chain

(linked V_H-V_L or single chain F_V). Both V_H and V_L may copy

natural monoclonal antibody sequences or one or both of the chains may comprise a CDR-FR construct of the type described in U.S. Patent 5,091,513 (the entire content of which is hereby incorporated herein by reference). The separate polypeptides analogous to the variable regions of the light and heavy chains are held together by a polypeptide linker. Methods of production of such single chain antibodies, particularly where the DNA encoding the polypeptide structures of the V_H and V_L chains are known, may be accomplished in accordance with the methods described, for example, in U.S. Patents 4,946,778, 5,091,513 and 5,096,815, the entire contents of each of which are hereby incorporated herein by reference.

[00146] An antibody is said to be "capable of binding" a molecule if it is capable of specifically reacting with the molecule to thereby bind the molecule to the antibody. The term "epitope" is meant to refer to that portion of any molecule capable of being bound by an antibody which can also be recognized by that antibody. Epitopes or "antigenic determinants" usually consist of chemically active surface groupings of molecules such as amino acids or sugar side chains and have specific three dimensional structural characteristics as well as specific charge characteristics.

[00147] Polyclonal antibodies are heterogeneous populations of antibody molecules derived from the sera of animals immunized with an antigen.

[00148] Monoclonal antibodies (mAbs) are a substantially homogeneous population of antibodies to specific antigens. MAbs may be obtained by methods known to those skilled in the art. See, for example Kohler et al., (1975); U.S. Patent No. 4,376,110; Harlow et al., (1988); and Colligan *et al.*, (1993), the entire contents of which references are incorporated entirely herein by reference. Such antibodies may be of any immunoglobulin class including IgG, IgM, IgE, IgA, and any subclass thereof. The hybridoma producing the mAbs of this invention may be cultivated *in vitro* or *in vivo*. High titers of mAbs can be obtained by *in vivo* production where cells from the individual hybridomas are injected intraperitoneally into pristane-primed Balb/c mice to produce ascites fluid containing high concentrations of the desired mAbs. MAbs of isotype IgM or IgG may be purified from such ascites fluids, or from culture supernatants, using column chromatography methods well known to those of skill in the art.

[00149] Chimeric antibodies are molecules, the different portions of which are derived from different animal species, such as those having a variable region derived from a murine mAb and a human immunoglobulin constant region. Chimeric antibodies are

primarily used to reduce immunogenicity during application and to increase yields in production, for example, where murine mAbs have higher yields from hybridomas but higher immunogenicity in humans, such that human/murine chimeric or humanized mAbs are used. Chimeric and humanized antibodies and methods for their production are well-known in the art, such as Cabilly et al., 1984; Morrison et al., 1984; Boulianne et al., 1984; Cabilly et al., 1984; Neuberger et al., 1985; Taniguchi et al., 1985; Morrison et al., 1986; Neuberger et al., 1986; Kudo et al., 1986; Morrison et al., 1986; Sahagan et al., 1986; Robinson et al., 1987; Liu et al., 1987; Sun et al., 1987; Better et al., 1988; and Harlow et al., 1988. These references are hereby incorporated herein by reference.

[00150] A "molecule which includes the antigen-binding portion of an antibody," is intended to include not only intact immunoglobulin molecules of any isotype and generated by any animal cell line or microorganism, or generated in vitro, such as by phage display technology for constructing recombinant antibodies, but also the antigen-binding reactive fraction thereof, including, but not limited to, the Fab fragment, the Fab' fragment, the $F(ab')_2$ fragment, the variable portion of the heavy and/or light chains thereof, and chimeric or single-chain antibodies incorporating such reactive fraction, or molecules developed to deliver therapeutic moieties by means of a portion

of the molecule containing such a reactive fraction. Such

molecules may be provided by any known technique, including, but

not limited to, enzymatic cleavage, peptide synthesis or

recombinant techniques.

[00151] The present invention also provides a pharmaceutical

composition containing a molecule which includes the antigen-

binding portion of an antibody raised against a polypeptide/

peptide of the present invention, and a pharmaceutically

acceptable, carrier, diluent, excipient or auxiliary agent. The

formulation of pharmaceutical compositions, which formulation is

conventionally used in a highly skilled art and which

compositions are suitable for its intended use as a therapeutic

for reducing the formation of amyloid fibrils and deposits, prion

deposits, amylin deposits, deposits containing α-synuclein, or

deposits of proteins with polyglutamine repeats, can be developed

with only routine experimentation by those of skill in the art.

[00152] According to the present invention, the molecule which

includes the antigen-binding portion of an antibody raised

against the immunogenic polypeptides/peptides of the present

invention can be administered to a subject in need thereof to

reduce the formation of amyloid fibrils and deposits, prion

deposits, amylin deposits, deposits containing α-synuclein, or

deposits of proteins containing polyglutamine repeats. The site

of administration, the dosage, and the schedule of administration

are determined according to well-established procedures used by those of skill in the art.

[00153] As shown in Example 5, in the case of antibodies against prions, those that have a K_D for PrP^{Sc} lower than 2, preferably lower than 0.5, even more preferably lower than 0.1 nM; a K_D for PrP^C lower than 20, preferably lower than 5, even more preferably lower than 1 nM; and/or a K_D for recPrP lower than 0.5, preferably lower than 0.2, even more preferably lower than 0.1 nM; are particularly preferred for vaccination or post-exposure prophylaxis against prion disease.

Testing of Peptides or Antibodies

[00154] The peptides and antibodies designed according to the present invention can be tested for various properties, including their secondary structure and their efficacy in reducing aggregation of, and eliciting immune responses directed against, peptides associated with the conformational disorders described herein. Exemplary techniques for testing are described below and in Example 1.

[00155] *Secondary Structure*. In one embodiment, a peptide preparation of the invention contains a relatively lower portion of β-sheets than a preparation of the disease-associated peptide. The proportion of β-sheets in a preparation can, as described in Example 1, be determined by known techniques such as circular

dichroism (CD; Soto et al., 1998 and 1996, Golabek et al., 1996),
or Fourier Transform InfraRed spectroscopy (FTIR; Aucouturier et
al., 1999).

[00156] *Aggregation, fibril formation, and/or amyloid
formation*. To study the tendency of a peptide preparation to
aggregate or form fibrils, various established methods can be
employed. For example, various concentrations of a peptide
preparation can be incubated in an aqueous solution, preferably a
buffer, for a predetermined time, and fibril formation thereafter
measured by a Thioflavine T fluorescent assay. Thioflavine T
binds specifically to amyloid and this binding produces a shift
in its emission spectrum and a fluorescent enhancement
proportional to the amount of amyloid formed (LeVine et al. 1993,
Castaño et al., 1995; Wisniewski et al., 1991; Wisniewski et al.,
1993 and 1994).

[00157] Alternative methods include spectrophotometric assays
based on the specific interaction of Congo red with amyloid
fibrils (Castaño et al., 1986), optionally in conjunction with
electron microscopic examination after negative staining (Castaño
et al., 1995; Wisniewsi et al., 1991; Wisniewski et al., 1993 and
Wisniewski et al., 1994), or sedimentation assays in which
soluble and aggregated peptide are separated by centrifugation,
and the concentration of peptide in solution analyzed by
electrophoresis or chromatography (Soto et al., 1995). The

application of these and other methods for the study of fibril

formation of Aβ-peptides are described in Example 1. By routine

experimentation, however, these and similar methods can be

modified to study fibril formation or aggregation of peptides or

variants of prion proteins, amylin, α-synuclein, and

polyglutamine repeats.

[00158] *Neurotoxicity*. Neurotoxicity of peptides and

antibodies can be evaluated by use of, for example, a standard

MTT assay as described by the manufacturer (Roche Molecular

Biochemicals, Indianapolis, IN) and a human neuroblastoma cell

line such as SK-N-SH). In addition, the MTT protocol described

in Example 1 for evaluation of Aβ peptides can be modified to

study neurotoxicity of variants of prion proteins, amylin, α-

synuclein, and polyglutamine repeats.

[00159] *Vaccine efficacy*. Evaluation or pre-clinical

optimization of peptide or antibody vaccines in eliciting an

immune response preventing, delaying, or reducing the formation

of the aggregates, deposits, or fibrils involving the peptides

associated with confirmational disordes can advantageously be

made in animal models. The animal studies can be designed to

evaluate preventive or prophylactic vaccination (i.e., peptide

administration before any symptom of disease) or treatment of an

already existing condition (i.e., peptide administration after a

disease symptom). Preferred, although nonlimiting, animal models

are transgenic or non-transgenic mice and rats, as well as

monkeys, dogs, cats, sheep and cattle. Preferably, the animals

develop a conformational disease spontaneously, or the

development of a conformational disease can be triggered, i.e.,

by adminstration of a disease agent (e.g., scrapie to induce

prion disease).

[00160] Several animal models have been established for the

study of conformational diseases and their progression. For

example, Example 1 describes the use of the transgenic mouse

model Tg2576 (Hsiao et al., 1996) to study vaccination against

amyloid deposits. These animals can develop Aβ plaques at 11-13

months of age. Prophylactic vaccination is preferably initiated

before any substantial amyloid burden has developed, and

typically repeated weekly, biweekly, or monthly, or a combination

thereof, with or without adjuvant. Other animal models for AD

include the double transgenic APP/PS1 model (Holcomb et al.,

1998).

[00161] Examples 3-5 describe the use of a CD-1 mouse model,

which is a strain of a wild-type mouse, for investigating the

delay of onset of prion disease when administering a recombinant

prion peptide or anti-prion antibodies before or after injection

of scrapie-infected mouse brain tissue. In this model, a

preparation of prion peptides or peptide variants, or anti-prion

antibodies, can be administered either before exposure to scrapie

for the study of immunization, after exposure to scrapie but before onset of prion disease to study prophylactic capabilities (i.e., to delay onset of disease); and after the onset of prion disease to evalue treatment potential. Several other mouse strains that are useful for the study of prion disease are knonw in the art.

[00162] As for the study of amylin peptide or antibody vaccines, older cats and monkeys spontaneously develop diabetes-associated islet amyloidosis (Howard,1978; de Koning, 1993; Yano et al., 1981; and O'Brien, 1986), and at least three transgenic mouse models have been developed (Couce et al., 1996; Fox et al., 1993; D'Allessio., 1994, and Höppener et al., 1993). These mice express the human gene for islet amyloid polypeptide (IAPP) but do not develop diabetes, and islet amyloidosis is rare (Höppener et al., 1993; Verchere et al., 1997). However, amylin deposition and diabetes can be induced in these mice by making them obese and by increasing insulin resistance by various approaches, such as by adding dietary fat (Verchere et al., 1996), by administering steroids or hormones (Couce et al., 1996), or by co-expression of obesity genes (Soeller et al., 1998; Vroom et al., 1999). These models can be used to determine if vaccination with amylin derivatives prevents and/or reverses amylin deposition. See also Jaikaran and Clark, 2001.

[00163] Several transgenic mouse models overexpressing human α-synuclein have been developed. Although none of these models have all the features of Parkinson's disease (PD), many exhibit synucleinopathy-induced neurodegeneration. The model that most closely recapitulates the α-synuclein pathology observed in PD, expresses human α-synuclein with the mutation A53T under the mouse prion promoter (Giasson et al., 2002; Lee et al., 2002). This is the only transgenic mouse model in which α-synuclein fibrils are observed, and it is also the only mammalian PD model that develops progressive neurodegeneration resulting in cell death. Other PD models containing Lewy body-like inclusions (fibrillar α-synuclein) include rats that have received the pesticide rotenone intravenously (Betarbet et al., 2002), and drosophila expressing human α-synuclein (Feany and Bender, 2000). Various other models that develop non-fibrillar or atypical α-synuclein inclusions can also be useful to test α-synclein derived immunization approaches. See, also Dawson et al. (2002).

[00164] For the study of immunization approaches to polyglutamine repeats, an animal model of Huntington's Disease (HD) can be employed. Two types of transgenic mouse models have been developed for HD (Rubinsztein, 2002): (1) transgenics in which the mutant human gene is randomly inserted into the mouse genome; and (2) 'knock-ins' in which the mutant gene is inserted

into the mouse huntingtin gene. The first mouse model of HD was developed by overexpression of exon 1 of the human huntingtin gene with long CAG-repeat expansions (Mangiarini et al., 1996). Several mouse models that recapitulate many aspects of HD, including the presence of huntingtin aggregates in the brain, have also been described (Hodgson et al., 1999; Schilling et al., 1999; Reddy et al., 1998; Yamamoto et al., 2000; Laforet et al., 2001; White et al., 1997; Shelbourne et al., 1999; Lin et al., 2001). In addition to these mouse models, drosophila models of polyglutamine expansion diseases have been developed (Kazemi-Esfarjani et al., 2000; Fernandez-Funez et al., 2000; Marsh et al., 2000; Warrick et al., 1999). See, also Rubinsztein DC, 2002.

[00165] Specific protocols for each type of peptide or antibody preparation and disease type can be designed using no more than routine experimentation combined with general knowledge in the art and the present disclosure. For example, Example 1 describes a vaccination protocol using 100 µg Aβ peptide variant per administration with or without Freund's complete or incomplete adjuvant, and Example 3 describes vaccination using bi-weekly injections of 50 µg recombinant PrP, administering Freund's complete adjuvant in conjunction with the first administration and Freund's incomplete for the subsequent ones. Typical vaccination protocols are also provided in Sigurdsson et al.,

(2001, Am J Pathol 2002, and 2002 (in press)). Similar experimental protocols can be used for immunizations in animal models for vaccination of diseases associated with amylin fibrils, α-synuclein fibrils and filaments, and protein aggregates containing polyglutamine repeats, e.g., huntingtin, although the adjuvant and dosages can be varied or optimized as appropriate.

[00166] Assessment of vaccination efficacy is conducted using standard methods such as histological examination using, for example, examination of sectioned tissues of interest, antibody staining techniques to visualize the extent of deposits or fibrils in selected tissues, ELISA methods for estimating plasma or tissue concentrations of the disease-associated peptide or endogenous antibodies directed to the disease-associated peptide, or testing whether deposits or aggregates of the disease-associated peptide are resistant to proteinase digestion.

[00167] Vaccine efficacy in preventing or delaying a neurodegenerative conformational disorder can also be evaluated by testing for motor coordination and/or cognitive capabilities at appropriate intervals during disease progression.

[00168] For example, locomotor activity of a vaccinated or control animal can be tested by putting the animal, typically a rodent, into a closed activity box for 5 minutes. The animal's activity in the box is detected by photoreceptors in the box, so

that whenever an animal crosses the receptor, an activity count is recorded. The activity box can record activity counts per minute. See, also, Sobotka et al., 1978.

[00169] Alternatively, the ability of the animal to cross a traverse beam can be evaluated. The animal is given 1 unscored training trial, preventing injury from falling by placing a soft cover underneath the beam. An animal that falls off is placed back into the position they maintained prior to the fall. After training, each animal is tested twice. Errors are defined as footslips and recorded both numerically and using Feeney scores. See, also, Quartermain et al., 2000.

[00170] Motor coordination can also be studied using a rotarod. The animal is placed onto a clean rod (diameter 3.6 cm) for 30 seconds. With each 30-sec interval, the rotation speed is increased incrementally. Total time (including the 30-sec on the quiescent rod) and RPM when the animal fell down is recorded. A soft cover is placed beneath the apparatus to prevent potential injury from falling. Each animal is tested thrice with an intertrial interval of fifteen minutes. See, also, Quartermain et al., 2000.

[00171] As for cognitive tests, animals can be randomly split into equivalent groups and then run on a series of cognitive tests such all groups receive each test in a different sequential order. Cognitive testing can be made in various settings known

in the art, e.g., radial arm mazes, linear mazes, water mazes, and goal boxes. For example, in a maze experiment, each animal can undergo a predetermined time of adaptation, consisting of 15 minutes free moving in the maze, with pieces of fruit loops in each (open) arm of the maze. Subjects are then exposed to doors. Animals are food deprived before the first adaptation with, for example, approximately ten percent body weight loss. Fruit loops are added to normal diet before deprivation schedule starts. Testing include recording correct and incorrect arms entered. Animals are placed in the center of the maze and all doors are opened. After entry into an arm, the animal must find and eat the reinforcer before the door in opened to re-enter the center of the maze. Testing ends when all arms are entered and reinforcers found. Re-entry into an arm constitutes an error. Total number of errors and time to enter all arms are recorded. Access to food is given for 3 - 4 hours (depending on age, body weight loss) daily. Radial arms mazes and other types of cognitive tests are described in Ammassari-Teule et al. (1985), Roullet et al. (1998), and Roullet et al. (1995).

[00172] Having now generally described the invention, the same will be more readily understood through reference to the following examples which are provided by way of illustration and is not intended to be limiting of the present invention.

EXAMPLE 1

[00173] The experiments in this example demonstrate that immunization in transgenic APP mice (Tg2576) for 7 months with a non-amyloidogenic, non-toxic Aβ homologous peptide reduced cortical and hippocampal brain amyloid burden by 89% (p = 0.0002) and 81% (p = 0.0001), respectively. Concurrently, brain levels of soluble Aβ1-42 were reduced by 57% (p = 0.0019). Ramified microglia expressing interleukin-1β associated with the Aβ plaques were absent in the immunized mice indicating reduced inflammation in these animals. The materials and methods used in the experiments in this example and the experimental results are presented below.

MATERIALS AND METHODS

Peptides

[00174] The peptides used (Aβ1-40, Aβ1-42, Aβ1-30-NH₂ (SEQ ID NO:1), and K6Aβ1-30-NH₂ (SEQ ID NO NO:6)) were synthesized at the Keck Foundation (Yale University, New Haven, CT), as described previously (Sigurdsson et al., 2000). Non-amyloidogenic peptides according to the present invention are synthesized using solid-phase tBOC (N-*tert*-butyloxycarbonyl) chemistry, purified by HPLC, and characterized by HPLC and laser desorption mass spectroscopy.

[00175] The peptide used for the immunizations, K6Aβ1-30-NH₂, maintains the two major immunogenic sites of Aβ peptides, which are residues 1-11 and 22-28 of Aβ1-42 based on the antigenic index of Jameson et al. (1998), and on preliminary results obtained in the laboratory of the present inventors. The Aβ1-30-NH₂ and K6Aβ1-30-NH₂ peptides were amidated at the C-terminus to further preserve their antigenicity.

Secondary structure studies

[00176] Secondary structure (α-helix, β-sheet, and random coil) of the peptides was evaluated by circular dichroism (CD) as described previously (Soto et al., 1998 and Soto et al., 1996). Results are expressed as molar ellipticity in units of deg cm^2 dmol^{-1}, and the data was analyzed by the Lincomb and CCA algorithms (Perczel et al., 1992) to obtain the percentages of different types of secondary structure.

[00177] While the secondary structure of the synthesized peptides was evaluated by circular dichroism (CD), it can also be evaluated by Fourier-Transform InfraRed spectroscopy (FTIR), using published protocols from Aucouturier et al. (1999). Although CD is sensitive to backbone conformation and FTIR is sensitive to the degree and strength of hydrogen bonding of amide groups (which is dependent of the structure), these two techniques ultimately give similar information: the percentages

of different secondary structure motifs, i.e., α-helix, β-sheet,

β-turn and random coil (Surewicz et al., 1993). CD is a very

well-established technique for studying the secondary structure

of proteins and peptides in solution, giving fairly accurate

estimations of the content of different structural motifs. A

major advantage of FTIR spectroscopy for structural

characterization is the lack of dependence on the physical state

of the sample. Samples may be examined as aqueous or organic

solutions, hydrated films, inhomogeneous dispersions, aggregated

materials or even proteins in solid state. Therefore, CD and

FTIR are complementary for studying the secondary structure of

peptides.

[00178] The experimental procedure for circular dichroism (CD)

is performed according to Golabek et al., (1996) and Soto et al.

(1996 and 1998) as follows: CD spectra of solutions cóntaining

synthetic peptides (1-5 µM in 300 µl of 10 mM sodium phosphate,

pH 7.2) is recorded in a Jasco J-720 spectropolarimeter at 25°C

using a 0.1 cm path-length cell with double distilled, deionized

water and TFE (spectroscopy grade) being used as solvents.

Calibration of the instrument is performed with an aqueous

solution of d-(+)-10-camphorsulfonic acid. Spectra is recorded

at 1 nm intervals over the wavelength range 180 to 260 nm and

buffer spectra obtained under identical conditions is subtracted.

[00179]   The experimental procedure for Fourier-Transform
InfraRed Spectroscopy according to Aucouturier et al. (1999) is
as follows: Solutions or suspensions containing soluble or
aggregated synthetic peptides (5-10 mg/ml) will be prepared in
H_2O and D_2O buffers at neutral pH, and 10 µl will be loaded into
an infrared cell with CaF_2 plates and 6 µm path-length spacer.
Spectra will be recorded with a Perkin Elmer model 2000 FTIR
spectrophotometer at 25°C, as described (Aucouturier et al.,
1999; Soto et al., 1995). For each spectrum, 1000 scans will be
collected in the single-beam mode with 2 cm^{-1} resolution and a 1
cm^{-1} interval from 4000 to 1000 cm^{-1}. Smoothing and Fourier
self-deconvolution will be applied to increase the spectral
resolution in the amide I region (1700 - 1600 cm^{-1}) and the
iterative fitting to Lorentzian line shapes will be carried out
to estimate the proportion of each secondary structural element.

Studies of amyloid fibril formation *in vitro*

[00180] Studies of amyloid fibril formation *in vitro* can be
performed using published protocols from the laboratory of the
present inventors (Castaño et al., 1995; Wisniewski et al., 1991;
Wisniewski et al., 1993 and Wisniewski et al., 1994). Aliquots
of the synthetic peptides at a concentration ranging between
25-250 µM, prepared in 0.1M Tris, pH 7.4, can be incubated for
different times, and their fibril formation compared to that of

Aβ1-28, Aβ1-40 and Aβ1-42. In this example, aliquots of the

peptides prepared in 0.1M Tris, pH 7.4, were incubated for

different times, and their fibril formation compared to that of

Aβ1-30-NH$_2$ and Aβ1-42. *In vitro* fibrillogenesis was evaluated by

a fluorometric assay based on the fluorescence emission by

thioflavine T, as previously described by the laboratory of the

present inventors (Soto et al., 1998 and Jameson et al., 1998).

Thioflavine T binds specifically to amyloid and this binding

procedures a shift in its emission spectrum and a fluorescent

enhancement proportional to the amount of amyloid formed (LeVine

et al. 1993).

[00181] Although not performed in this example, *in vitro*

fibrillogenesis can also be evaluated by three other different

methods:

[00182] (A) A spectrophotométric assay based on the specific

interaction of Congo red with amyloid fibrils. After the

incubation period, 2 µl of Congo red (1.5 mg/ml) will be added to

each sample and incubated in the dark for 1 h. The samples will

then be centrifuged at 15,000 rpm for 10 min and the absorbance

of the supernatant measured at 490 nm. The amount of amyloid

formed is directly proportional to the decrease in the

supernatant absorbance (Castaño et al., 1986).

[00183] (B) A sedimentation assay will be used as described

(Soto et al., 1995). Briefly, samples will be centrifuged at

15,000 rpm for 10 min to separate the soluble and aggregated peptide. The amount of material in solution will be analyzed by microbore HPLC using a reverse phase Vydac C4 column and a linear gradient of 3-70% acetonitrile. The percentage of aggregated peptide will be estimated by comparing the area of the peak corresponding to the soluble peptide in each incubated sample with an identical control of non-incubated sample.

[00184] (C) Additional characterization of fibrillogenesis will be performed by Congo red staining and electron microscopic examination after negative staining (Castaño et al., 1995; Wisniewsi et al., 1991; Wisniewski et al., 1993 and Wisniewski et al., 1994). For electron microscopy, the incubated samples of peptides will be placed on carbon formar-coated 300-mesh nickel grids and stained for 60 seconds with 2% uranyl acetate under a vapor of 2% glutaraldehyde. Grids will be visualized on a Zeiss EM 10 electron microscope at 80 kV. For Congo red staining, the incubated peptides will be placed onto gelatin-coated glass microscope slides and air-dried at 37°C. The slices will then be immersed in 0.2% Congo red dissolved in 80% aqueous ethanol saturated with NaCl for 60 min at room temperature, washed three times with water and visualized by polarized light microscopy.

Neurotoxicity

[00185] The potential neurotoxicity of K6Aβ1-30-NH$_2$ (1-100 μM) was evaluated at 2 and 6 days in a human neuroblastoma cell line (SK-N-SH) using the standard MTT assay as described by the manufacturer (Roche Molecular Biochemicals, Indianapolis, IN). Aβ 1-30-NH$_2$, Aβ1-40 and Aβ1-42 were used as control peptides. Briefly, cells were plated at 10,000 cells/100 μl culture medium per well in flat bottom, 96 well microtiter plates. The cells were allowed to attach to the plate overnight in an incubator (37°C, 5.0% CO$_2$), and then 10 μl of freshly prepared peptide solution (in nanopure H$_2$O) was added. Aβ1-42 was only partially soluble at 100 μM and was, therefore, added as a suspension at that concentration. Subsequent steps were as described in the assay protocol.

Animals

[00186] The vaccination was performed in the Tg2576 APP mouse model developed by Karen Hsiao et al. (1996). These mice develop Aβ plaques as early as at 11-13 months of age. This model was chosen over the double Tg APP/PS1 model (Holcomb et al., 1998) because the age of onset and progression of Aβ deposition in the single Tg APP mice more closely resembles that of AD. Age-matched vehicle-treated Tg mice and non-Tg littermates receiving K6Aβ1-30-NH2 were used as controls, and the animals received their

first injection at 11-13 months, at which time few plaques should already be present. Four mice were in each group. The animals were maintained on a 12 h light-dark cycle, and had access to food and water ad libitum. The animal care was in accordance with institutional guidelines.

[00187] Vaccine Administration: K6Aβ1-30-NH$_2$ was supplied as trifluoroacetic acid (TFA) salt. The immunization procedure was performed as previously described by Schenk et al. (1999) except that the peptide was not incubated overnight at 37°C before injection. Briefly, the peptide was dissolved in PBS at a concentration of 2 mg/ml and then mixed 1:1 (v/v) with the adjuvant or PBS. Complete Freund's adjuvant was used for the first injection, incomplete Freund's adjuvant for the next 3 injections, and PBS from the 5th injection forward. The mice received a subcutaneous injection of 100 μl of the mixture (i.e., 100 μg/100 μl) followed by a second injection two weeks later, and then monthly thereafter.

[00188] Antibody Titers: Antibody titers were determined by serial dilutions of sera using an ELISA assay as described previously (Jimenez-Huete et al., 1998), where Aβ or its derivative is coated onto microtiter wells. The titer, defined as the dilution yielding 50% of the maximum signal, was detected by a goat anti-mouse IgG linked to a horseradish peroxidase

(Amersham Pharmacia Biotech, Piscataway, NJ), and tetramethyl

benzidine (Pierce, Rockford, IL) was the substrate.

[00189] <u>Histology</u>: Mice were anesthetized with sodium

pentobarbital (150 mg/kg, i.p.), perfused transaortically with

phosphate buffer and the brains processed as previously described

(Sigurdsson et al., 1996). The right hemisphere was immersion

fixed in periodate-lysine-paraformaldehyde, whereas the left

hemisphere was snap frozen for measurements of Aβ levels using

established ELISA methods (Mehta et al., 1998 and Mehta et al.,

2000). Serial coronal sections (40 μm) were cut and five series

of sections at 0.2 mm intervals were saved for histological

analysis of 1) 6E10, 2) Congo red, 3) Interleukin-1β/OX42/tomato

lectin, 4) GFAP, and 5) cresyl violet stained sections. 6E10

recognizes Aβ and stains both pre-amyloid and Aβ plaques (Kim et

al., 1990). Congo red staining was performed to identify amyloid

lesions in these animals. GFAP is a component of the glial

intermediate filaments that form part of the cytoskeleton and is

found predominantly in astrocytes. Microglia appear to be the

major source of interleukin-1 (IL-1) within the CNS (Schobitz et

al., 1994), and OX-42 recognizes CD11b on microglia, a rat

equivalent of the human C3bi receptor (Robinson et al., 1986).

Tomato lectin binds to poly-N acetyl lactosamine residues and has

in neural tissue specific affinity for microglial cells (Acarin

et al., 1994). Both astrocytes and microglia are associated with

Aβ deposits. Staining with cresyl violet was performed to determine if the immunization was causing neuronal shrinkage and/or cell loss in these animals. Following sectioning, the series were placed in ethylene glycol cryoprotectant and stored at -20°C until used.

[00190] Cresyl violet and Congo red: Mounted sections were defatted in xylene and hydrated in a gradient of ethyl alcohol and water series. Staining was performed as previously described (Sigurdsson et al., 1996 and 1997 and Soto et al., 1998)

[00191] 6E10, GFAP, IL-1β and OX-42: Staining was performed as previously described (Sigurdsson et al., 1996 and Soto et al., 1998). Briefly, sections were incubated in 6E10 (kindly provided by Richard Kascsak, Institute for Basic Research) primary antibody that selectively binds to human Aβ at a 1:1000 dilution. A mouse on mouse immunodetection kit (Vector Laboratories, Burlingame, CA) was used where the anti-mouse IgG secondary antibody was used at a 1:2000 dilution. GFAP (1:500; Dako, Denmark), IL-1β (1:250; Endogen, Rockford, IL) and OX-42 (1:250; Biosource Int., Camarillo, CA) staining was performed the same way as the 6E10 staining, except the secondary antibody was diluted 1:1300. The sections were reacted in 3,3'-diaminobenzidine tetrahydrochloride (DAB) with or without nickel ammonium sulfate (Ni) intensification. For double labeling of IL-1β and Aβ plaques, sections were first stained for IL-1β (DAB/Ni;

black) where peroxidase was the enzyme. The plaques (6E10) were then stained using the Vector Red Alkaline Phosphatase Substrate Kit I (Vector).

[00192] Tomato Lectin: Sections removed from the cryoprotectant were washed in PBS, 0.3% Triton-X-100 in PBS (PBS-Tx) and then incubated for 30 minutes in 0.3% hydrogen peroxide in PBS to quench endogenous peroxidase activity. Following 2 hours incubation with tomato lectin (10 µg/ml PBS; Vector), sections were washed in PBS-Tx and then reacted with avidin-horseradish peroxidase (Vector) for one hour. Subsequent steps were as those used for the antibody staining.

[00193] Image Analysis: Immunohistochemistry of tissue sections was quantified with a Bioquant image analysis system, and unbiased sampling was used (West et al., 1999). All procedures were performed by an individual blind to the experimental condition of the study. Cortical area analyzed was dorsomedially from the cingulate cortex and extended ventrolaterally to the rhinal fissure within the right hemisphere. The area of the grid was 800 x 800 μm^2 and amyloid load was measured in 10 frames per mouse (each: 640 x 480 μm^2), chosen randomly. Hippocampal measurements were performed on the entire hippocampus in a similar manner as the cortical analysis. The Aβ burden is defined as the percent of area in the measurement field occupied by reaction product.

[00194] <u>Sandwich ELISA Assay for Soluble Aβ Levels:</u> Prior to

extraction of Aβ from brain tissue, 10% (w/v) homogenates were

prepared in tissue homogenization buffer (20 mM Tris pH 7.4, 250

mM sucrose, 1 mM EDTA, 1 mM EGTA). Immediately before use, 1/100

volume of 100 mM phenylmethylsulfonyl fluoride stock solution (in

ethanol) and 1/1000 volume of LAP (5 mg each of leupeptin,

antipain and pepstatin Aβ per ml of N-N-dimethylformamide) were

added to the homogenization buffer. The homogenate was then

thoroughly mixed with an equal volume of 0.4% diethylamine/100mM

NaCl, then spun at 135,000 x g for one hour at 4°C, and

subsequently neutralized with 1/10 volume 0.5 M Tris, pH 6.8. The

samples were then aliquoted, flash frozen on dry ice, and stored

at -80°C until loaded onto plates. Soluble Aβ levels were

measured in the left hemisphere using monoclonal antibody 6E10

(specific to an epitope present on 1-16 amino acid residues of

Aβ), rabbit antiserum R162 (specific for Aβ40) and rabbit

antiserum 165 (specific for Aβ42) in a double antibody sandwich

ELISA as described previously (Mehta et al., 1998 and 2000). The

optical density (OD) was measured at 450 nm in a microELISA

reader. The relationship between OD and Aβ40 or Aβ42

concentrations was determined by a four-parameter logistic log

function. Nonlinear curve fitting was performed with KlinetiCalc

program (Biotek Instruments, Inc. Winooski, VT) to convert OD of

plasma to estimated concentrations. All samples were coded, and

the investigators were blinded to group assignment until levels
were measured and recorded. The detection limit of the assay is
10 pg/ml for Aβ40 and Aβ42. The percent coefficient of variation
normally ranges from 8 to 14% (inter-assay) and 10 to 18% (intra-
assay).

[00195] Data Analysis: The cell culture data was analyzed by
one-way ANOVA, followed by a Dunnett's test for post hoc analysis
(GraphPad Prism 3.0). An unbiased stereological image analysis
system (Bioquant, R&M Biometrics Inc., Nashville, TN) was used to
determine the amyloid burden in 6E10 stained brain sections. The
data for the amyloid burden and the levels of soluble Aβ within
the brain were analyzed by a Student's t-test, two-tailed.

RESULTS

[00196] Before conducting the vaccination study it was
necessary to confirm that the prototype peptide, KKKKKK-Aβ1-30-
NH2, had indeed less β-sheet structure, reduced fibrillogenicity
compared to Aβ 1-42, and that it was non-toxic in neuronal
culture. The secondary structure of these peptides was determined
by circular dichroism (CD), and their ability to form amyloid
fibrils by a thioflavin-T fluorometric assay. An additional
control peptide was Aβ1-30-NH2.

[00197] CD Assay: Compounds with high β-sheet content are more
toxic and more likely to form fibrils than compounds with low β-

sheet content (Pike et al., 1991). The peptide with the
polylysine at the N-terminus had much less β-sheet content that
the amidated Aβ1-30 or Aβ1-42 (Table 1).

[00198] The (K)$_6$-Aβ1-30-NH$_2$ peptide also does not form fibrils
following incubation at 37°C for at least 15 days. This data
clearly shows that the addition of polylysine at the N-terminus
alters the peptide so that the β-sheet content is much lower then
either Aβ1-42 or Aβ1-30. In addition, the β-sheet content of the
(K)$_6$-Aβ1-30-NH$_2$ peptide does not increase with time. The β-sheet
content of Aβ1-42 increased to 55% after 96 hr., while that of
(K)$_6$-Aβ1-30-NH$_2$ stayed at 16-18%.

Table 1

Time (hr)	Aβ1-42			Aβ1-30-NH$_2$			(K)$_6$-Aβ1-30-NH$_2$		
	alpha	beta-sheet	random	Alpha	beta-sheet	random	alpha	beta-sheet	random
0	9	36	55	5	37	58	2	18	79
24	9	40	51	8	36	56	5	16	78
96	5	55	40	7	49	44	34	16	50

[00199] Thioflavin T assay: Aβ1-42 was already fibrillar at t =
0, whereas Aβ1-30-NH$_2$ gradually formed fibrils over time (Figure
1). The relatively high degree of thioflavin T staining of the
Aβ1-30-NH$_2$ versus Aβ1-42 after 6 days reflects the known batch-

to-batch variability of Aβ peptide fibril formation (Soto et al., 1995), as well as some degree of pellet formation by the Aβ1-42 with prolonged incubation. K6Aβ1-30-NH$_2$ did not form fibrils following incubation at 37°C for at least 15 days.

[00200] Neurotoxicity: To further assess the safety of this vaccination approach the neurotoxicity of K6Aβ1-30-NH$_2$ was determined. K6Aβ1-30-NH$_2$ had no effect on cell viability at 2 days and was slightly trophic at 6 days ($p < 0.05$), whereas Aβ1-40 and Aβ1-42 were toxic ($p < 0.05-0.001$) to the human neuroblastoma cells (SK-N-SH), compared to vehicle group, as determined by the MTT assay (Figure 2A and B). During the incubation period, aggregates were visible under the microscope only in culture wells containing Aβ1-42 (10-100 μM).

[00201] Antibody Titer: Tg2576 and their non-Tg littermates were vaccinated with K6Aβ1-30-NH$_2$ or vehicle. Almost all the mice developed antibodies against the immunogen (K6Aβ1-30-NH$_2$), that cross-reacted with Aβ1-40 and Aβ1-42. The titer, defined as the dilution yielding 50% of the maximum signal, ranged from a few hundreds to several thousands. Vehicle treated animals injected with the adjuvant and PBS did not develop antibodies against these three peptides. Non-transgenic mice had generally higher titer against all 3 peptides, and the polyclonal antibodies had higher avidity for the immunogen compared to Aβ1-40 and Aβ1-42.

These findings are as expected because the immunogen is based on the human sequence of Aβ which differs in 3 amino acids from the mouse Aβ (Johnstone et al., 1991), and K6Aβ1-30-NH$_2$ that elicited the immune response should have more binding motifs for antibodies than the intact Aβ peptides.

[00202] <u>Amyloid Burden and Associated Histopathology:</u> The mice were killed at 18-20 months of age after 7 months treatment, and their right hemisphere was processed for histology as described (Sigurdsson et al., 1996). The brain sections were stained with cresyl violet, Congo red, tomato lectin and with antibodies against: 1) human Aβ (6E10); microglia (OX-42; IL-1β); and GFAP (anti-GFAP). Following K6Aβ1-30-NH$_2$ vaccination, cortical and hippocampal amyloid burden in the Tg mice was reduced by 89% and 81%, respectively (Figures 3A, 3B; 4A, 4B), as determined by stereological techniques. The total number of Congo red positive amyloid deposits was reduced in the immunized animals; however, the percentage of Aβ-immunoreactive lesions that were Congo red positive appeared to remain the same as in the non-immunized Tg mice. The clearance of the amyloid deposits appeared to be similar in other brain regions. Selected brain sections from a control mouse with high amyloid burden and an immunized mouse with reduced amyloid burden were stained with sera from several immunized and control mice, whose antibody titer ranged from zero to three thousand. As expected, with increasing titer more

plaques were stained and the pattern was similar in both mice.

There was no obvious difference between the Tg treatment groups

in cresyl violet staining. Reactive astrocytes were observed

associated with all amyloid plaques. Since the vehicle-treated Tg

mice had a higher plaque burden, they had more clusters of

astrocytes than immunized Tg mice. OX-42 staining of ramified

rather than phagocytic (ameboid) microglia was predominantly

observed associated with plaques. To verify that this lack of

microglial phagocytes was not due to downregulation of the CD11b

receptor, the binding motif of OX-42 (Robinson et al., 1986),

sections from all treatment groups were stained with tomato

lectin. This particular lectin binds to poly-N-acetyl lactosamine

residues found predominantly in ramified and phagocytic

microglial cells, in addition to endothelial- and ependymal cells

(Acarin et al., 1994). These two latter cell types were stained

in all the mice. The microglial lectin staining resembled the OX-

42 staining. In other words, in both immunized and control Tg

groups, the microglia did not have phagocytic morphology and

number of ramified microglial processes per plaque appeared to be

similar between immunized and non-immunized mice. On the other

hand, IL-1β staining of ramified microglial cells was prominent

surrounding the Aβ plaques in the control Tg mice (Figure 3C),

whereas virtually no IL-1β staining was observed in the immunized

mice (Figure 3D). Significantly, there was no indication of

glomerulonephritis in hemotoxylin/eosin stained kidney sections

from the K6Aβ1-30-NH$_2$ treated mice, suggesting that the mice had

not developed an autoimmune disorder.

[00203] <u>Soluble Aβ by ELISA:</u> Measurements of soluble Aβ levels

were performed on the left hemisphere of the mice whose right

hemisphere was used for histology. Soluble Aβ1-42 was reduced by

57% following vaccination with K6Aβ1-30-NH$_2$ for 7 months (p =

0.0019), compared to control group (Figure 4C). Although there

was a trend for reduced levels of soluble total Aβ and Aβ1-40 in

the K6Aβ1-30 treated group, the values were not significantly

different from the vehicle group.

[00204] Overall, immunization in Tg APP mice with non-

amyloidogenic/non toxic (low β-sheet content) Aβ homologous

peptide results in a similar reduction of amyloid burden as

observed by Schenk et al. (1999) where they used a

fibrillar/toxic (high β-sheet content) Aβ1-42.

DISCUSSION

[00205] These findings demonstrate that Aβ aggregates/fibrils

are not necessary to elicit a sufficient immune response that

results in clearance of Aβ plaques. The use of non-fibrillar/non-

toxic Aβ homologous peptides, such as K6Aβ1-30-NH$_2$, is a safer

vaccination approach for humans.

[00206] The mechanism of the vaccination-induced reduction in
cerebral amyloid burden is not fully understood. However, based
on the passive vaccination study by Bard et al. (2000) it is
likely that antibodies have a pivotal role. Interestingly, they
demonstrated that there was no correlation between antibody
efficacy and affinity for soluble Aβ or binding to aggregated
synthetic Aβ peptide. Effective antibodies were, however, able to
bind to plaques in unfixed brain sections. Janus et al. (2000),
using the same protocol as Schenk et al. (1999) observed that the
sera from Aβ-immunized mice preferentially stained dense core
plaques rather than diffuse Aβ deposits suggesting that the
antibodies may have a higher affinity for β-sheet Aβ. Based on
these somewhat contradictory findings, more studies are needed on
Aβ-antibody interactions that may give insight into the mechanism
of antibody-mediated Aβ clearance. It is unlikely that these
antibodies are affecting the production of Aβ because they do not
recognize APP (Weiner et al., 2000). It is more probable that the
antibodies enhance clearance of Aβ through microglial activation
following antibody binding to Aβ plaques (Schenk et al., 1999 and
Bard et al., 2000). Their effect may also in part be due to
binding to soluble Aβ within the brain, that alters the
equilibrium between deposited Aβ vs. soluble Aβ. Given the
numerous reports that show that Aβ can bi-directionally cross the
blood brain barrier (Zlokovic et al., 1993; Maness et al., 1994;

- 110 -

Martel et al., 1996; Poduslo et al., 1997 and 1999; Mackic et al., 1998; Shibata et al., 2000 and Ji et al., 2001) the vaccination effect may be in part mediated through binding of the antibodies to soluble Aβ in peripheral fluids. Subsequent reduction in peripheral Aβ levels may alter the equilibrium between Aβ found within and outside the CNS that may result in efflux of Aβ out of the CNS. A recent report shows that in the Tg2576 mice, plasma levels of Aβ decrease as cerebral plaque burden increases (Kawarabayashi et al., 2001). This suggests an interaction between these two compartments that can be manipulated.

[00207] Interestingly, in the behavioral vaccination study by Morgan et al. (2000), they observed a partial reversal in cognitive deficits in APP/PS1 mice although cerebral amyloid burden as measured by immunohistochemistry was not significantly reduced. As pointed out by Morgan et al. (2000), soluble Aβ has been proposed to cause synapse loss in APP Tg mice, as some Tg lines have reduced synaptophysin staining in the dentate gyrus without Aβ deposits (Mucke et al., 2000). Therefore, a possible explanation for the cognitive improvement in the immunized mice in the absence of reduced plaque burden, was a decrease in soluble Aβ, although this potential connection was not measured in their study (Morgan et al., 2000). The results obtained in the laboratory of the present inventors show that following 7 months

treatment, the 81-89% reduction in amyloid plaque burden is associated with a 57% reduction in soluble Aβ1-42 within the brain, whereas the reduction in soluble total Aβ and Aβ1-40 was not significantly different from the control group. In other words, soluble Aβ is reduced less than plaque Aβ. However, detailed time course studies must be performed to determine further any changes in the equilibrium between soluble- and plaque Aβ. These findings indirectly demonstrate the importance of Aβ1-42 for plaque maintenance. Overall, it is likely that several different mechanisms may result in reduction of cerebral amyloid burden, depending on the animal model and the properties of the peptide used for immunization.

[00208] Numerous studies have suggested that amyloid deposition can activate inflammatory cascades in the brain, such as increased IL-1 production associated with neuronal injury and death (Sigurdsson et al., 1996 and Akiyama et al., 2000). It is possible that our immunization with Aβ homologous peptides could also stimulate such negative inflammatory pathways, along with amyloid reduction. However, few phagocytic microglia were observed in our immunized animals, as identified by OX-42 immunoreactivity or tomato lectin binding. This is not surprising because after 7 months treatment most of the plaques have been cleared. Furthermore, in the immunized group of mice microglial IL-1β staining was virtually absent, whereas numerous ramified

IL-1β positive microglia were associated with the plaques in the

control Tg group. The laboratory of the present inventors have

previously reported a similar lack of IL-1β staining in a rat

model of cerebral amyloidosis following treatment with a β-sheet

breaker peptide (Sigurdssone et al., 2000). However, in that

acute study (16 days) this effect was associated with extensive

increase in phagocytic OX-42 staining, indicating that phagocytes

do not express IL-1β. The current observations from the

experiments in this example may suggest that an important effect

of the immunization is reduced inflammation within the brain.

EXAMPLE 2

MATERIALS AND METHODS

Peptides

[00209] The peptides used (Aβ1-40, Aβ1-42, Aβ1-30-NH$_2$, K6Aβ1-30-

NH$_2$, Aβ1-30-K6 (SEQ ID NO:11), Aβ1-30-NH$_2$(EE$_{18,19}$) (SEQ ID NO:12),

Aβ1-30-NH$_2$(DD$_{18,19}$) (SEQ ID NO:13) were synthesized at the Keck

Foundation (Yale University, New Haven, CT), as described

previously (Sigurdsson et al., 2000). The Aβ homologous peptides

maintain the two major immunogenic sites of Aβ peptides (residues

1-11 and 22-28 of Aβ1-42 based on the antigenic index of Jameson

et al. (1998) and on preliminary results obtained in the

laboratory of the present inventors), while being non-fibrillar

and non-toxic.

Study of amyloid fibril formation *in vitro* and neurotoxicity

[00210] The experiments were performed as described in Example 1.

[00211] <u>Data Analysis</u>: The cell culture data was analyzed by one-way ANOVA, followed by a Newman Keuls' test for post hoc analysis (GraphPad Prism 3.0).

RESULTS

[00212] Thioflavin T assay: $A\beta1-42$ was already fibrillar at $t = 0$, whereas $A\beta1-30-NH_2$ and $A\beta1-40$ gradually formed fibrils over time (Figure 5). $A\beta1-30K6$ was slightly fibrillogenic but $A\beta1-30-NH_2(EE_{18,19})$ and $A\beta1-30-NH_2(DD_{18,19})$ did not form fibrils following incubation at 37°C for at least 15 days.

[00213] <u>Neurotoxicity</u>: To further assess the safety of this vaccination approach the neurotoxicity of the peptides was determined (Figures 6A and 6B). Treatment effect was observed both at 2 and 6 days ($p < 0.0001$). The control peptides $A\beta1-40$ and $A\beta1-42$ were toxic ($p < 0.01-0.001$) to the human neuroblastoma cells (SK-N-SH), compared to vehicle group, as determined by the MTT assay. $K6A\beta-30-NH_2$ had no effect on cell viability at 2 days and was slightly trophic at 6 days ($p < 0.001$), and the highest dose (100 μM) of $A\beta1-30K6$ was slightly toxic following 2 days treatment but not at 6 days. During the incubation period,

aggregates were visible under the microscope only in culture wells containing Aβ1-42 (10-100 μM). These Aβ homologous peptides according to the present invention do not form fibrils and are non-toxic in human neuronal culture. Overall, this approach has a much lower risk of leading to toxic effects in humans, than the use of Aβ1-40/42.

EXAMPLE 3

[00214] Prion infections do not illicit a classical immune response; however, transport of prions from the periphery to the central nervous system is critically dependent on the lymphoreticular system. In this example, the present inventors sought to determine how active immunity against PrP would influence progression of disease. The experiments described herein show that vaccination with recombinant mouse prion protein (recPrP) delays the onset of prion disease in mice.

Methods

[00215] Twenty female CD-1 mice, 2-3 months of age, were immunized with mouse recPrP. For the first injection, the recPrP (Brown et al., 1999) (1 mg/ml in 0.5 M urea) was mixed with an equal volume of complete Freund's adjuvant immediately before subcutaneous administration (50 μg recPrP/100 μl). Twenty control mice received the adjuvant plus vehicle. Subsequent

immunizations were performed at 2 weeks intervals in incomplete

Freund's adjuvant. Fourteen weeks following the first

vaccination the mice were bled and the PrP antibody titer was

determined by ELISA (Fig. 7). The mice were subsequently divided

into two groups matched for their titer to recPrP and were

inoculated intraperitoneally with a brain homogenate of the

mouse-adapted scrapie strain 139A at a 10-fold (Fig. 8A) and a

1000-fold (Fig. 8B) dilution. The control mice were also divided

into two groups that received either the 10-or 1000-fold dilution

of same 139A inoculum. The immunization was continued thereafter

at monthly intervals until the first mice showed clinical

symptoms of scrapie. The mice were sacrificed when they scored

positive by observers blinded to the experimental status of the

animals for three consecutive weeks (Soto et al., 2000). The

diagnosis of prion disease was confirmed by staining brain

sections with a polyclonal anti-PrP antibody (anti-PrP 78295) and

by the detection of proteinase K resistant PrP on Western blots

(Kascsak et al., 1997).

Results

[00216] As depicted in Figure 9, the mice immunized with the

recPrP had a delayed onset of scrapie symptoms (two-way ANOVA,

$p=0.0005$ for vaccination effect). The Newman-Keuls post hoc test

revealed a more pronounced effect at the 10 fold dilution

(p=0.001, two-tailed). This is most likely related to a significantly higher average titer of anti-PrP antibodies at the time of sacrifice in the group receiving a lower dilution of PrP^{Sc} (p<0.05). A higher anti-PrP^{C} antibody titer, in vaccinated animals, correlated with a longer incubation time in both the PrP^{Sc} inoculated mouse groups (lower dilution group: r^2=0.4389, p=0.0052; higher dilution group: r^2=0.6786, p<0.0001).

[00217] While not being limited to any particular theory, it is possible that antibody binding to PrP^{C} and/or PrP^{Sc} may interfere with PrP^{Sc} mediated conversion of PrP^{C} to PrP^{Sc} and thereby delay the onset of clinical symptoms. Recent *in vitro* studies support this view (Enari et al., 2001; Peretz et al., 2001).

[00218] Furthermore, overexpression of recPrP in E. coli leads to the formation of inclusion bodies that stain positive for Congo red, indicating a potential toxic property. This toxic property would not be exhibited by a PrP peptide substituted according to the invention.

EXAMPLE 4

[00219] This example describes the application of recPrP as a rescue treatment, i.e., administration of recPrP after exposure to scrapie. See, also, Sigurdsson et al. (Am J Pathol 2002).

Methods

[00220] The experiment was performed similarly to the prophylactic treatment mouse group described in Example 3, except for immunization with recPrP being started 24 hours after intraperitoneal inoculation with mouse-adapted scrapie strain 139A. As before, there were two groups of 10 female CD-1 mice each at 2 to 3 months of age, which received either a 10-fold or 1000-fold dilution of the 139A brain homogenate inoculum intraperitoneally, followed in this case by the first recPrP injection in complete Freund's adjuvant 24 hours later. There were also two control groups of 10 CD-1 mice, which received either the 10-fold or 1000-fold dilution of the same 139A brain homogenate inoculum intraperitoneally, but followed by injection of adjuvant plus vehicle (0.5 mol/L urea). The rest of the protocol was identical to the prophylactic treatment mouse group in the 10-fold PrP^{Sc} dilution group (see above).

Results

[00221] As expected, the effects of the treatment were somewhat less pronounced in the rescue mouse group, compared to the prophylactically treated mice (see, Figure 2A in Sigurdsson et al., Am J Pathol 2002). No significant group difference was observed in disease onset in mice receiving the 10-fold dilution of the brain inoculum (days to sacrifice, 192 ± 5 days (control)

versus 190 ± 5 days), although the levels of antibodies against

PrP^C correlated with disease onset (r^2 = 0.279, P < 0.017).

However, at the 1000-fold dilution, a delay in symptoms was

observed in the vaccinated mice (days to sacrifice, 210 ± 3 days

(control) versus 222 ± 4 days; P < 0.018, t-test, one-tailed). As

with the prophylactic treatment, the anti-PrP C antibody levels

in the immunized mice correlated with a longer incubation time

(see, Figure 2B in Sigurdsson et al. (Am J Pathol 2002); r2 =

0.772, P < 0.0001). Histological and Western blot evaluations of

all of the brains of the treated and control groups did not

reveal any apparent differences in the degree of spongiform

change or PrP^{Sc} levels at the time of sacrifice in either the

prophylactic or rescue treatment mouse experiments. Hence,

immunization delayed PrP^{Sc} propagation, but ultimately similar

pathology and levels of PrP^{Sc} were obtained in treatment and

control groups.

EXAMPLE 5

[00222] This example describes the application of anti-PrP

antibodies for prophylaxis following prion exposure in a mouse

model.

[00223] One hundred female CD-1 mice, 2 months of age, were

inoculated intraperitoneally (i.p.) with a brain homogenate of

the mouse-adapted scrapie strain 139A at a 10-fold or a 1000-fold

dilution. This represents a well established model of prion

disease in mice, which leads to CNS scrapie infection and death

in all cases, if the disease is allowed to progress (Sigurdsson

et al., Am J Pathol 2002; Soto et al., 2000).

[00224] Immediately following inoculation, the mice received

i.p. injection (50 µg/500 µl phosphate buffered saline) of

monoclonal anti-PrP antibodies (8F9, 8B4 or 8H4; ten mice per

group at each dilution of scrapie) (Liu et al., 2001). Twenty

mice received pooled mouse IgG (Sigma, St. Louis, MO) and a

further 20 received no injections. Subsequent passive

immunizations were continued on a weekly basis until the week of

sacrifice, which occurred when the mice scored positive for three

consecutive weeks on a behavioral test for prion disease. The

symptoms of scrapie were determined by a test of motor

coordination, performed by observers blinded to the experimental

status of the animals, using an established protocol (Sigurdsson

et al., Am J Pathol 2002; Soto et al., 2000). The diagnosis of

prion disease was confirmed by staining brain sections with

cresyl violet, immunostaining with a polyclonal anti-PrP antibody

(anti-PrP 78295) and by the detection of proteinase K resistant

PrP from brain and spleen on Western blots as previously

described (Sigurdsson et al., Am J Pathol 2002; Soto et al.,

2000).

[00225] The epitopes of the antibodies are as follows: 8B4 (residues 34-52), 8H4 (175-185) and 8F9(205-233) (Liu et al., (2001)). The affinity of the antibodies was determined by ELISA against mouse recombinant PrP, mouse PrP^C and mouse PrP^{Sc}. The PrP^{Sc} was prepared from ME7 infected mouse brains using an established protocol (Diedrich et al., (1991)). The PrP^C was prepared by treating the purified PrP^{Sc} with formic acid so that it reverted to proteinase K digestion sensitivity (Kascak et al., (1997)). Under these ELISA conditions, the KD of binding of 8F9 was lower compared to 8H4 or 8B4 (2.10 versus 0.04 and 0.05 nM, respectively for PrP^{Sc}; 43 versus 0.09 and 6.5 nM for PrP^C, respectively; 0.7 versus 0.07 and 0.08 nM, respectively for recPrP). The duration the anti-PrP antibodies were present in the serum following i.p. injection was assessed by bleeding the mice and testing the serum by ELISA for reactivity against recombinant PrP. Ten minutes following i.p. injection of the monoclonal antibody, its concentration in the serum was 20 µg/ml (±4 µg/ml); 4 h following i.p. injection the antibodies were no longer detectable in the serum by ELISA.

[00226] Two-way analysis of variance revealed a significant treatment-($P < 0.0001$) and dilution effect ($P < 0.000001$). The 8B4 and 8H4 antibodies were more effective than the 8F9 antibody; this is probably related to the higher affinities of these antibodies to PrP^C and PrP^{Sc}. Within the 10-fold PrPSc dilution

group, Newman-Keuls post hoc analysis showed that the incubation

times for both the non-injected and the IgG control groups was

significantly different from the 8B4 (P < 0.05) and the 8H4 group

(P < 0.05). Within the 1000-fold PrP^{Sc} dilution group, the IgG

and non-injected control groups significantly differed from the

8B4 group (P < 0.001 and P < 0.05, respectively, one-tailed). Ten

percent of the 8B4-injected animals did not develop disease. As

anticipated, no apparent differences were observed in the animals

that became ill, between the treatment and control groups in

terms of the scrapie associated pathology and the PrP^{Sc} levels as

they were sacrificed at equivalent time points after becoming

clinically ill.

[00227] These findings demonstrate the therapeutic effect of

anti-prion antibodies for post-exposure prophylaxis for prion

infection.

EXAMPLE 6

[00228] This example describes vaccination of Tg2576 APP mice

(see Example 1) with K6Aβ1-30-NH2 and Aβ1-42 using aluminum

adjuvants, and behavioral analysis of vaccinated animals.

Testing of Aluminum Adjuvants

[00229] In general, the type of aluminum adjuvant chosen is the

one that allows the greatest adsorption of the antigen onto the

adjuvant. The pI (isoelectric point) for Aβ1-42 is 5.42 and the

peptide has a negative charge at physiological pH, whereas the pI

for K6Aβ1-30-NH2 is 9.9 and the peptide has a positive charge at

physiological pH. Alhydrogel® (Superfos Biosector, Denmark) has a

positive charge under biological conditions and was therefore

selected for testing with Aβ1-42. Adju-Phos® (Superfos Biosector)

has a negative charge under the same conditions, and was

therefore selected for K6Aβ1-30-NH2.

[00230] Briefly, 30 μl of Alhydrogel® or Adju-Phos® were added

to 30 μg of peptide, the mixture was vortexed and then rotated

overnight at 4°C. Following centrifugation, the peptide

concentration in the supernatant was determined by a Comassie

Plus® (Pierce) protein assay according to the manfacturer's

instructions. Table 2 shows the adsorption of peptides onto the

adjuvants, verifying that Alhydrogel® and Adju-Phos® successfully

adsorb Aβ1-42 and K6Aβ1-30-NH2, respectively.

Table 2

	Adsorbed K6Aβ1-30-NH$_2$	Adsorbed Aβ1-42
Alhydrogel®	82.7%	98.1%
Adju-Phos®	97.5%	Not-tested

Animal Vaccination

[00231] Tg2576 mice, 3.5 to 4 months old, were injected subcutaneously with either 100 μg K6Aβ1-30-NH2 in 100 μl Adju-Phos® ("tg-k6" group); 100 μg Aβ1-42 in 100 μl Alhydrogel® ("tg-ab42" group); or Adju-Phos® or Alhydrogel® alone (100 μl, "tg-ah" or "tg-ap" group, respectively). Non-transgenic littermate mice injected with 100 μg K6Aβ1-30-NH2 in 100 μl Adju-Phos® were used as a control ("ntg-k6" group). All animals received the first 5 injections at 2-week intervals, with monthly injections thereafter.

Antibody Titers

[00232] The first group of mice were bled two weeks after the 5th injection, and the antibody titer to K6Aβ1-30, Aβ1-42, and Aβ1-40 determined by ELISA (see Example 1 for assay description). A sample of the results are visualized in Figure 11. Figure 11A-C shows that sera from animals vaccinated with K6Aβ1-30-NH2 contain antibodies which bind to antigen (Fig. 11A), as well as crossreacting antibodies binding to Aβ1-42 (Fig. 11B) and Aβ1-40 (Fig. 11C). The titers in mice injected with K6Aβ1-30-NH2 were reasonably high and in a similar range as in mice injected with the same peptide together with Freund's adjuvant (see Example 1). Figure 12 shows that sera from animals immunized with Aβ1-42

contain antibodies which bind to antigen (Fig. 12A), as well as crossreacting antibodies binding to K6Aβ1-30-NH2 (Fig. 12B) and Aβ1-40 (Fig. 12C). In general, the mice injected with Aβ1-42 had similar titers as the mice injected with the Aβ derivative. Table 3 shows titer data (EC50 values) for individual mice (one per row) in this group.

Table 3

Group	Aβ1-40 Titer	Aβ1-42 Titer	K6Aβ1-30 Titer
ntg-k6	9949	9951	7356
ntg-k6	74	86	120
ntg-k6	2	121	91
ntg-k6	5480	4333	5839
ntg-k6	124	35	166
ntg-k6	166	223	ND
ntg-k6	65	41	ND
tg-k6	14	1	104
tg-k6	2	1	131
tg-k6	327	171	146
tg-k6	215	694	1174
tg-k6	33	228	2816
tg-ab42	237	128	52
tg-ab42	3033	3187	2614

tg-ab42	6	275	443
tg-ab42	0	80	68
tg-ab42	38	130	562
tg-ab42	2427	3240	1341
tg-ah	208	289	508
tg-ap	283	587	395
tg-ah	89	76	80
tg-ah	103	102	351
tg-ap	388	671	528

ND = Not Done

[00233] A second group of animals was bled 2 weeks after the 5th injection and 28 weeks after the 5th injection (i.e., about four months after the first blood samples). Table 4 shows titer data (EC50 values) for individual mice in this group.

Table 4

Group	Aβ1-40 Titer		Aβ1-42 Titer		K6Aβ1-30 Titer	
	2w	28w	2w	28w	2w	28w
tg-k6	171	1	401	ND	732	ND
tg-k6	1	2	8	1	324	2118
tg-k6	112	431	113	1	279	1387
tg-k6	302	48	237	78	159	30

tg-k6	6	162	145	151	151	1378
tg-k6	121	ND	347	ND	730	ND
tg-k6	9	632	87	1398	180	4341
tg-k6	17	1	1	104	48	90
tg-k6	0	11	0	61	3	117
tg-ah	1	1	0	ND	1122	10
tg-ah	ND	7	ND	3	ND	148
tg-ah	977	3	9	1	1473	1227
tg-ah	7	ND	270	1	841	150
tg-ah	1	ND	2	ND	2	ND
tg-ah	5	8	1	1	3	271
tg-ah	4	1	0	139	1	209
tg-ah	1	1158	4	130	1	464
tg-ap	ND	3	ND	1	ND	3
tg-ap	0	4	0	3	4	4
tg-ap	4	3	1	252	2	505
tg-k6	0	3	0	3	205	1564

ND = Not Done

[00234] In these experiments, it was found that non-transgenic mice had higher titer against the antigen than transgenic mice. This was expected because the antigen contains the human sequence of Aβ which is foreign to the non-transgenic mice. It was also expected that the mice generally had higher titer against the

antigen compared to related peptides. Moreover, analysis of the distribution of titer levels showed that about 1/3 of the mice had high titers, 1/3 had medium titers and 1/3 had low titers. This is similar to what has been observed in rabbits used for polyclonal antibody production.

[00235] Determination of the amyloid burden in these animals and correlation between amyloid burden and titers will show the level of titer necessary to result in amyloid clearance.

Behavioral Studies - Methods

[00236] The following behavioral tests were performed when the animals were 3-4 months and 11 months of age. For statistical analysis of the results, ANOVA followed by Newman-Keuls post hoc test was applied.

Motor Coordination Tests

[00237] *Locomotor Activity.* After 15 minutes of room adaptation, animals were put into closed activity box for 5 minutes. Main room light was turned on throughout the adaptation and the test. The box consists of photoreceptors and whenever an animal crosses the receptor, an activity count is recorded. The activity box records activity counts per minute.

[00238] *Traverse Beam.* After 15 minutes room adaptation, with the big room light on, all animals were given 1 unscored training

trial (animals were put into closed goal box for 1 minute and put on the opposite end of the beam thereafter, facing the cardboard wall). Training trial ended when the animal entered the goal box. To prevent injury from falling, a soft yellow colored cover was put underneath the beam. Animals that fell off were placed back into the position they maintained prior to the fall. After training, each animal was tested twice. Errors were defined as footslips and recorded both numerically and using Feeney scores.

[00239] *Rotarod.* Following 15 min room adaptation (lights on), the animal was placed onto the rod (diameter 3.6 cm) for 30 seconds. With each 30-sec interval, the rotation speed was increased by 1/4 grade on the machine's scale. Total time (including the 30-sec on the quiescent rod) and RPM when the animal fell down were recorded. The rod was cleaned with a dry cloth before each animal started its trial. A soft yellow cover was placed beneath the apparatus to prevent potential injury from falling. Each animal was tested thrice with an intertrial interval of fifteen minutes.

Cognitive Tests

[00240] Animals were randomly split into three equivalent groups and then run on each test such that all three groups received each test in a different sequential order.

[00241] *Radial Arm Maze.* Animals were kept in the test room throughout the experiment, behind a cover to prevent view of the apparatus and room. Each animal underwent 2 days of adaptation, consisting of 15 minutes exploration in the maze (2 subjects at a time), with 3 pieces of fruit loops in each arm. Subjects were exposed to doors only on day 2. Animals, having approximately ten percent body weight loss, were food deprived 1 day before adaptation session I. Fruit loops were added to normal diet 5 days before deprivation schedule started. Animals entered and exited the maze through the center. Testing included recording correct and incorrect arms entered. Animals were placed in the center of the maze and all doors were opened. After entry into an arm, the animal had to find and eat the reinforcer before the door was opened for the animal to re-enter the center of the maze. Testing ended when all eight arms had been entered and reinforecers found. Re-entry into an arm constituted an error. Total number of errors and time to enter all eight arms were recorded. Access to food was given for 3 - 4 hours (depending on age, body weight loss) daily, and the apparatus was cleaned with 95 % ethanol after each animal. Arms were cleaned with dry cloth after each cage.

[00242] *Morris Water Maze.* Apparatus: A round, white plastic pool (d = 75 cm, h = 14.5 cm) was filled with water 5 cm below the top edge. The water was rendered opaque adding 120 ml of

white finger paint (Rich Art, Northvale, NJ). Water was kept at

30 degree Celsius.

[00243] For straight alley swimming, animals were placed into

one end of a straight alley constructed from wood and had to find

the hidden platform placed at the other end. After finding the

platform the animals were allowed to sit there for 15 s. There

was an inter-trial interval of 7 to 8 minutes upon which the

animal was given the subsequent trial. This was repeated for 6

trials. Animals were removed from the platform using a wooden

plank to avoid handling and extraneous stress.

[00244] For visual platform testing, on the second day, animals

had to locate a visible platform (salient cue on top, platform

0.5 cm above water line). The location of each platform was

varied five times. Animals were started at random points in the

maze (maximum latency 60 s). If the animal could not find the

platform within 60 seconds, it was gently led to it. After

locating the platform, the animal was allowed to remain on the

platform for 15 seconds. Furthermore, the pool was shunted in

order to avoid any extra-maze cues.

[00245] For acquisition in invisible platform testing, animals

had to locate the invisible platform (1.0 cm below waterline).

Three daily trials (different start positions) were administered

for a total of six days. Each animal received three trials with

an inter-trial interval of 7 to 8 minutes. If the animal could

not locate the platform within sixty seconds, it was led to it

and allowed to remain there for 15 seconds. After each trial,

the animal was placed under a heating lamp until the next trial.

[00246] Finally, a probe trial procedure was carried out in

order to assess retention of the location of the platform. Animal

was placed into the center of the pool (no platform) and allowed

to swim for 60 s. % distance scores for each quadrant were

obtained and compared across groups.

[00247] *Linear maze.* An outline of the maze is shown in Figure

13. Briefly, in the maze of Fig. 13, during adaptation, the

alleys are unblocked, and the animal must run from north goal box

to south and back and learn to drink the reinforcer; sucrose

solution. During testing, the west alleys are blocked, and the

animal must learn to use the east alleys. Error zones are

dotted. Testing begins in the south goal box, and the animal

must go to the north goal box (returning to the goal box of

origin is also an error). Time and error is recorded. The

criterion is shuttling with zero errors in 4 out of 5 trials.

For reversal, after having reached the criterion, the alleys that

are blocked are reversed (i.e., animals having learned the east

route must now learn the west route).

[00248] Animals were water deprived throughout the experiment

and kept at a body weight loss of 12 ± 2 % (= 40 min/day access

to H_2O). A 4 % sucrose solution (dissolved in regular tap H_2O and

dyed with non-toxic food coloring to increase contrast) was used as a reinforcer (made freshly 1/week). Floor and walls of the maze were cleaned using 50 % ethanol before each trial. Valves were rinsed with 12 cc 95 % ethanol, followed by 48°C distilled H_2O whenever the reinforcer was changed.

[00249] For step 1, adaptation, following 24 hours of water deprivation, animals were individually adapted to the maze for 8 min and trained to shuttle between the goal boxes. Animals were placed in the goal box and allowed time to explore the maze. None of the alleys were blocked. Adaptation procedure was repeated for two days to ensure that animals shuttled from the north end to south end as well as drank the sucrose solution.

[00250] For step 2, acquisition, alleys located on the same side of the maze (E or W) were randomly blocked for each animal during acquisition. Mice were given a maximum of daily 20 trials until they reached a criterion of 4/5 errorless trials. An error was recorded when the animal looked into a blocked alley or changed direction. Maximum interval/session: 20 min.

[00251] For step 3, reversal, when an animal reached criterion (4/5 errorless), the session was finished. The next day, for animals who successfully had reached criterion, the blocked alleys were reversed (i. e., from E to W or vice versa). The reversal procedure was repeated twice, so that in total each animal had 1 acquisition and 2 reversals scores.

Behavioral Studies - Results

[00252] Figures 14A, 14B, and 14C show the results obtained in
the respective groups when the animals were about 3-4 months of
age. The figures show the results from locomotor activity,
spontaneous avoidance, and passive avoidance, Figs. 14A-C,
respectively. Significant differences were observed between the
groups in locomotor activity (Two-way ANOVA: treatment; $p=0.046$,
time; $p<0.0001$). Neuman Keuls post hoc analysis revealed
significant differences between tg-ah/ap vs. nontg-k6 ($p=0.022$)
and tg-k6 ($p=0.027$). These results suggest that K6Aβ1-30-NH2 has
an acute effect, reducing the locomotor activity of tg mice to
that of their non-tg littermates.

[00253] Figures 15A-N show the results obtained in the
respective animal groups at 11 months of age. The figures show
the results from testing of locomotor activity (Fig. 15A), and
traverse beam (Figs. 15B and 15C), rotarod (Fig. 15D), radial arm
maze (Figs. 15E and 15F), straight alley channel (Fig. 15G),
visible platform (Figs. 15H and 15I), Morris water maze (hidden
platform) (Figs. 15J and 15K), probe trial (Figs. 15L and 15M),
and linear maze (Fig. 15N) skills. The y-axis in Figs. 15B and
15C represents Feeney scores and the total number of footslips,
respectively.

[00254] In the radial arm maze test (Fig. 15F), signficant differences were found between the ntg-k6 versus tg-ah/ap groups (p=0.04), tg-ab42 versus tg-ah/ap (p=0.03), and tg-k6 versus tg-ah/ap (p=0.01), showing that animals vaccinated with tg-ab42 or tg-k6 had the radial arm maze skills of the positive control, i.e., the non-transgenic animals. Significant treatment effects were also observed in the rotarod (Two-way ANOVA: treatment; p=0.025; trials; p<0.0001). Neuman Keuls post hoc analysis revealed a significant difference between tg-42 and tg-ap/ah groups (p=0.035).

*　　　　　*　　　　　*

[00255]　Having now fully described this invention, it will be appreciated by those skilled in the art that the same can be performed within a wide range of equivalent parameters, concentrations, and conditions without departing from the spirit and scope of the invention and without undue experimentation.

[00256]　While this invention has been described in connection with specific embodiments thereof, it will be understood that it is capable of further modifications. This application is intended to cover any variations, uses, or adaptations of the inventions following, in general, the principles of the invention and including such departures from the present disclosure as come

within known or customary practice within the art to which the invention pertains and as may be applied to the essential features hereinbefore set forth as follows in the scope of the appended claims.

[00257] All references cited herein, including journal articles or abstracts, published or corresponding U.S. or foreign patent applications, issued U.S. or foreign patents, or any other references, are entirely incorporated by reference herein, including all data, tables, figures, and text presented in the cited references. Additionally, the entire contents of the references cited within the references cited herein are also entirely incorporated by references.

[00258] Reference to known method steps, conventional methods steps, known methods or conventional methods is not in any way an admission that any aspect, description or embodiment of the present invention is disclosed, taught or suggested in the relevant art.

[00259] The foregoing description of the specific embodiments will so fully reveal the general nature of the invention that others can, by applying knowledge within the skill of the art (including the contents of the references cited herein), readily modify and/or adapt for various applications such specific embodiments, without undue experimentation, without departing from the general concept of the present invention. Therefore,

such adaptations and modifications are intended to be within the

meaning and range of equivalents of the disclosed embodiments,

based on the teaching and guidance presented herein. It is to be

understood that the phraseology or terminology herein is for the

purpose of description and not of limitation, such that the

terminology or phraseology of the present specification is to be

interpreted by the skilled artisan in light of the teachings and

guidance presented herein, in combination with the knowledge of

one of ordinary skill in the art.

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WHAT IS CLAIMED IS:

1. An isolated peptide represented by the formula
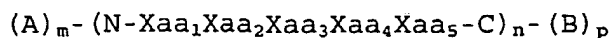
$$(A)_m-(N-Xaa_1Xaa_2Xaa_3Xaa_4Xaa_5-C)_n-(B)_p$$

wherein: m is 0, 4, 5, 6, 7, 8, 9, or 10;

p is 0, 4, 5, 6, 7, 8, 9, or 10;

A is Lys or Asp;

B is Lys or Asp;

n is 1 or 2;

N is residues 1-16 of SEQ ID NO:1;

C is residues 22-30 of SEQ ID NO:1;

Xaa_1, Xaa_2, Xaa_3, Xaa_4, and Xaa_5 are Leu, Val, Phe, Phe, and Ala, respectively, in which either

(A) at least one of residues Xaa_1, Xaa_2, Xaa_3, Xaa_4, and Xaa_5 is substituted with Lys, Asp, or Glu, all residues of the peptide are D-amino acid residues, and the amino acid sequence of the peptide is a member of the group consisting of SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:9, and SEQ ID NO:10; or

(B) at least one of residues Xaa_1, Xaa_2, Xaa_3, Xaa_4, and Xaa_5 is substituted with Pro, Gly, or Ser, all residues of said peptide are either all L-amino acid residues or all D-amino acid residues, and the amino acid sequence of the peptide is a member

- 154 -

of the group consisting of SEQ ID NO:15, SEQ ID NO:16, SEQ ID

NO:17, SEQ ID NO:18, SEQ ID NO:19, and SEQ ID NO:20; or

(C) zero residue is substituted, all amino acids are D-

amino acids, and either or both of m and p is not zero.

2.   The peptide of claim 1, wherein p is 0.

3.   The peptide of claim 2, wherein the C-terminus of

said peptide is amidated.

4.   The peptide of claim 2, wherein the amino acid

sequence of said peptide is SEQ ID NO:2 with all residues being

D-amino acid residues.

5.   The peptide of claim 2, wherein the amino acid

sequence of said peptide is SEQ ID NO:3 with all residues being

D-amino acid residues.

6.   The peptide of claim 2, wherein the amino acid

sequence of said peptide is SEQ ID NO:15 with all residues being

either all L-amino acid residues or all D-amino acid residues.

7.   The peptide of claim 2, wherein the amino acid

sequence of said peptide is SEQ ID NO:16 with all residues being

either all L-amino acid residues or all D-amino acid residues.

8.   The peptide of claim 1, wherein m is 0.

9.   The peptide of claim 8, wherein the amino acid

sequence of said peptide is SEQ ID NO:4 with all residues being

D-amino acid residues.

10.   The peptide of claim 8, wherein the amino acid sequence of said peptide is SEQ ID NO:5 with all residues being D-amino acid residues.

11.   The peptide of claim 8, wherein the amino acid sequence of said peptide is SEQ ID NO:17 with all residues being either all L-amino acid residues or all D-amino acid residues.

12.   The peptide of claim 8, wherein the amino acid sequence of said peptide is SEQ ID NO:18 with all residues being either all L-amino acid residues or all D-amino acid residues.

13.   The peptide of claim 1, wherein m is not zero and p is not zero.

14.   The peptide of claim 13, wherein the amino acid sequence of said peptide is selected from the group consisting of SEQ ID NO:9 and SEQ ID NO:10 with all residues being D-amino acid residues.

15.   The peptide of claim 13, wherein the amino acid sequence of said peptide is selected from the group consisting of SEQ ID NO:19 and SEQ ID NO:20 with all residues being either all L-amino acid residues or all D-amino acid residues.

16.   The peptide of claim 1, wherein one, two, three, four, or five residues of Xaa_1, Xaa_2, Xaa_3, Xaa_4, and Xaa_5 are substituted.

17.   A conjugate of the peptide of claim 1 cross-linked to a polymer molecule.

18.   The conjugate according to claim 17, wherein said polymer molecule is a peptide comprising a promiscuous T helper cell epitope.

19.   An immunizing composition, comprising an immunizing effective amount of the peptide of claim 1 or a conjugate thereof, and a pharmaceutically acceptable carrier, excipient, diluent, adjuvant or auxiliary agent.

20.   A method for inducing an immune response to amyloid β peptides and amyloid deposits, comprising administering the immunizing composition according to claim 19 to a subject in need thereof.

21.   A method of reducing amyloidosis comprising administering the immunizing composition according to claim 19 to a subject in need thereof, thereby reducing amyloidosis.

22.   A molecule which includes the antigen-binding portion of an antibody raised against the peptide of claim 1.

23.   The molecule according to claim 22, which is selected from the group consisting of monoclonal antibody, single chain antibody, and humanized antibody.

24.   A pharmaceutical composition, comprising a molecule according to claim 22 and a pharmaceutically acceptable carrier, diluent, excipient or auxiliary agent.

25. A method for reducing the formation of amyloid fibrils and deposits, comprising administering a molecule according to claim 22 to a subject in need thereof.

26. A method of reducing amyloidosis, comprising administering the pharmaceutical composition according to claim 24 to a subject in need thereof, thereby reducing amyloidosis.

27. An isolated polypeptide or peptide represented by:

(A) SEQ ID NO:32;

(B) a segment of SEQ ID NO:32, comprising residues 90 to 144 of SEQ ID NO:32, alone or in which either a polyaspartate or polylysine of 4 to 10 residues in length is joined to said segment at the N-terminus and/or C-terminus of said segment; or

(C) the formula

$(A)_m-(N-Xaa_1Xaa_2GlyGlyLeuGlyGlyXaa_3Xaa_4Xaa_5-C)_n-(B)_p$

wherein: m is 0, 4, 5, 6, 7, 8, 9, or 10;

p is 0, 4, 5, 6, 7, 8, 9, or 10;

A is Lys or Asp;

B is Lys or Asp;

n is 1 or 2;

N is residues 90-120 of SEQ ID NO:21;

C is residues 131-144 of SEQ ID NO:21;

Xaa_1, Xaa_2, Xaa_3, Xaa_4, and Xaa_5 are Val, Val, Tyr, Met, and Leu, respectively, in which zero or one to five of residues Xaa_1, Xaa_2, Xaa_3, Xaa_4, and Xaa_5 is substituted with Pro, Asp, Glu, Lys, Gly, or Ser; and

when zero residue is substituted, then either or both of m and p is not zero.

28.   The polypeptide or peptide of claim 27, wherein all residues are D-amino residues.

29.   The polypeptide or peptide of claim 27, wherein four or five of residues Xaa_1, Xaa_2, Xaa_3, Xaa_4, and Xaa_5 are substituted.

30.   The polypeptide or peptide of claim 27, which is SEQ ID NO:32.

31.   The polypeptide or peptide of claim 27, which is a segment of SEQ ID NO:32, comprising residues 90 to 144 of SEQ ID NO:32, alone or in which either a polyaspartate or polylysine of 4 to 10 residues in length is joined to said segment at the N-terminus and/or C-terminus of said segment.

32.   The polypeptide or peptide of claim 27, which is represented by the formula
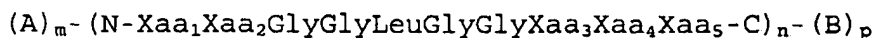
$(A)_m$-$(N$-$Xaa_1Xaa_2GlyGlyLeuGlyGlyXaa_3Xaa_4Xaa_5$-$C)_n$-$(B)_p$

wherein: m is 0, 4, 5, 6, 7, 8, 9, or 10;

p is 0, 4, 5, 6, 7, 8, 9, or 10;

A is Lys or Asp;

B is Lys or Asp;

n is 1 or 2;

N is residues 90-120 of SEQ ID NO:21;

C is residues 131-144 of SEQ ID NO:21;

Xaa_1, Xaa_2, Xaa_3, Xaa_4, and Xaa_5 are Val, Val, Tyr, Met, and Leu, respectively, in which zero or one to five of residues Xaa_1, Xaa_2, Xaa_3, Xaa_4, and Xaa_5 is substituted with Pro, Asp, Glu, Lys, Gly, or Ser,

wherein when zero residue is substituted, then either or both of m and p is not zero.

33.   The polypeptide or peptide of claim 30, wherein p is 0.

34.   The polypeptide or peptide of claim 33, wherein the C-terminus is amidated.

35.   The polypeptide or peptide of claim 33, wherein the amino acid sequence of said polypeptide or peptide is selected from the group consisting of SEQ ID NO:34 and SEQ ID NO:35.

36.   The polypeptide or peptide of claim 32, wherein m is 0.

37.   The polypeptide or peptide of claim 36, wherein the amino acid sequence of said polypeptide or peptide is selected from the group consisting of SEQ ID NO:36 and SEQ ID NO:37.

38.   The polypeptide or peptide of claim 32, wherein m
is not zero and p is not zero.

39.   The polypeptide or peptide of claim 38, wherein
the amino acid sequence of said polypeptide or peptide is
selected from the group consisting of SEQ ID NO:38 and SEQ ID
NO:39.

40.   A conjugate of the polypeptide or peptide of claim
27 cross-linked to a polymer molecule.

41.   The conjugate of claim 40, wherein said polymer
molecule is a peptide comprising a promiscuous T helper cell
epitope.

42.   An immunizing composition, comprising an
immunizing effective amount of the polypeptide or peptide of
claim 27 or a conjugate thereof, and a pharmaceutically
acceptable carrier, excipient, diluent, adjuvant, or auxiliary
agent.

43.   A method for inducing an immune response to prion
protein and prion deposits, comprising administering to a human
subject in need thereof either the immunizing composition of
claim 42 or an immunizing composition comprising a prion protein
or a fragment thereof from a mammalian species that does not
transmit prion disease to humans, which prion protein or a
fragment thereof may be modified at either or both termini or at

the corresponding conserved amino acid residues according to the polypeptide or peptide of the immunizing composition of claim 42.

44.   The method of claim 43, wherein the mammalian species is selected from the group consisting of mouse, rat, sheep, goat, Syrian hamster, mink, and greater kudu.

45.   A molecule which includes the antigen-binding portion of an antibody raised against the polypeptide or peptide of claim 27.

46.   The molecule of claim 45 which is selected from the group consisting of monoclonal antibody, single chain antibody, and humanized antibody.

47.   A pharmaceutical composition, comprising the molecule of claim 45 and a pharmaceutically acceptable carrier, diluent, excipient or auxiliary agent.

48.   A method for reducing the formation of the abnormal PrP^{sc} form of prion protein, comprising administering the molecule of claim 45 to a human subject in need thereof.

49.   An isolated polypeptide or peptide represented by:

(A) SEQ ID NO:33;

(B) a segment of the sequence of SEQ ID NO:33, comprising residues 93 to 156 of SEQ ID NO:33, alone or in which either a polyaspartate or polylysine of 4 to 10 residues in

length is joined to said segment at the N-terminus and/or C-terminus of said segment; or

(C) the formula

$(A)_m$-$(N$-$Xaa_1Xaa_2GlyGlyLeuGlyGlyXaa_3Xaa_4Xaa_5$- $C)_n$-$(B)_p$

wherein: m is 0, 4, 5, 6, 7, 8, 9, or 10;

p is 0, 4, 5, 6, 7, 8, 9, or 10;

A is Lys or Asp;

B is Lys or Asp;

n is 1 or 2;

N is residues 93-131 of SEQ ID NO:30;

C is residues 142-156 of SEQ ID NO:30;

Xaa_1, Xaa_2, Xaa_3, Xaa_4, Xaa_5 are Val, Val, Tyr, Met, and Leu, respectively, in which zero or one to five of residues Xaa_1, Xaa_2, Xaa_3, Xaa_4, Xaa_5 is substituted with Pro, Asp, Glu, Lys, Gly, or Ser; and

when zero residue is substituted, then either or both of m and p is not zero.

50.   The polypeptide or peptide of claim 49 wherein all residues are D-amino residues.

51.   The polypeptide or peptide of claim 49, wherein four or five of residues Xaa_1, Xaa_2, Xaa_3, Xaa_4, and Xaa_5 is substituted.

52.   The polypeptide or peptide of claim 49, which is represented by SEQ ID NO:33.

53. The polypeptide or peptide of claim 49, which is represented by a segment of the sequence of SEQ ID NO:33, comprising residues 93 to 156 of SEQ ID NO:33, alone or in which either a polyaspartate or polylysine of 4 to 10 residues in length is joined to said segment at the N-terminus and/or C-terminus of said segment.

54. The polypeptide or peptide of claim 49, which is represented by the formula
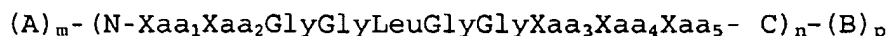
$(A)_m - (N-Xaa_1Xaa_2GlyGlyLeuGlyGlyXaa_3Xaa_4Xaa_5 - C)_n - (B)_p$

wherein: m is 0, 4, 5, 6, 7, 8, 9, or 10;

p is 0, 4, 5, 6, 7, 8, 9, or 10;

A is Lys or Asp;

B is Lys or Asp;

n is 1 or 2;

N is residues 93-131 of SEQ ID NO:30;

C is residues 142-156 of SEQ ID NO:30;

Xaa_1, Xaa_2, Xaa_3, Xaa_4, Xaa_5 are Val, Val, Tyr, Met, and Leu, respectively, in which zero or one to five of residues Xaa_1, Xaa_2, Xaa_3, Xaa_4, Xaa_5 is substituted with Pro, Asp, Glu, Lys, Gly, or Ser,

wherein when zero residue is substituted, then either or both of m and p is not zero.

55. The polypeptide or peptide of claim 54, wherein p is 0.

56.   The polypeptide or peptide of claim 55, wherein the C-terminus is amidated.

57.   The polypeptide or peptide of claim 55, wherein the amino acid sequence of said polypeptide or peptide is selected from the group consisting of SEQ ID NO:40 and SEQ ID NO:41.

58.   The polypeptide or peptide of claim 54, wherein m is 0.

59.   The polypeptide or peptide of claim 57, wherein the amino acid sequence of said polypeptide or peptide is selected from the group consisting of SEQ ID NO:42 and SEQ ID NO:43.

60.   The polypeptide or peptide of claim 54, wherein m is not zero and p is not zero.

61.   The polypeptide or peptide of claim 60, wherein the amino acid sequence of said polypeptide or peptide is selected from the group consisting of SEQ ID NO:44 and SEQ ID NO:45.

62.   A conjugate of the polypeptide or peptide of claim 49 cross-linked to a polymer molecule.

63.   The conjugate of claim 62, wherein said polymer molecule is a peptide comprising a promiscuous T helper cell epitope.

64. An immunizing composition, comprising an
immunizing effective amount of the polypeptide or peptide of
claim 49 or a conjugate thereof, and a pharmaceutically
acceptable carrier, excipient, diluent, adjuvant, or auxiliary
agent.

65. A method for inducing an immune response to prion
protein and prion deposits, comprising administering to a cow in
need thereof either the immunizing composition of claim 64 or an
immunizing composition comprising a prion protein or a fragment
thereof from a mammalian species that does not transmit prion
disease to cows, which prion protein or fragment thereof may be
modified at either or both termini or at the corresponding
conserved amino acid residues according to the isolated
polypeptide or peptide of the immunizing composition of claim 64.

66. A molecule which includes the antigen-binding
portion of an antibody raised against the polypeptide or peptide
of claim 49.

67. The molecule of claim 66 which is selected from
the group consisting of monoclonal antibody, single chain
antibody, and humanized antibody.

68. A pharmaceutical composition, comprising the
molecule of claim 66 and a pharmaceutically acceptable carrier,
diluent, excipient or auxiliary agent.

69. A method for reducing the formation of the abnormal PrP^{sc} form of prion protein, comprising administering the molecule of claim 66 to a cow in need thereof.

70. An isolated peptide represented by the formula
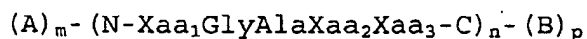
$$(A)_m-(N-Xaa_1GlyAlaXaa_2Xaa_3-C)_n-(B)_p$$

wherein: m is 0, 4, 5, 6, 7, 8, 9, or 10;

p is 0, 4, 5, 6, 7, 8, 9, or 10;

A is Lys or Asp;

B is Lys or Asp;

n is 1 or 2;

N is residues 1-22 of SEQ ID NO:46;

C is residues 28-37 of SEQ ID NO:46;

Xaa$_1$, Xaa$_2$, and Xaa$_3$ are Phe, Ile, and Leu, respectively, in which zero, one, two or three of residues Xaa$_1$, Xaa$_2$, and Xaa$_3$ is substituted with Pro, Asp, Glu, Lys, Gly, or Ser; and

when zero residues is substituted, then either or both m and p is not zero .

71. The peptide of claim 70, wherein all residues are D-amino residues.

72. The peptide of claim 70, wherein p is 0.

73. The peptide of claim 72, wherein the amino acid sequence of said peptide is selected from the group consisting of SEQ ID NO:48 and SEQ ID NO:49.

74. The peptide of claim 70, wherein m is 0.

75. The peptide of claim 74, wherein the amino acid sequence of said peptide is selected from the group consisting of SEQ ID NO:50 and SEQ ID NO:51.

76. The peptide of claim 70, wherein m is not zero and p is not zero.

77. The peptide of claim 76, wherein the amino acid sequence of said peptide is selected from the group consisting of SEQ ID NO:52 and SEQ ID NO:53.

78. A conjugate of the peptide of claim 70 cross-linked to a polymer molecule.

79. The conjugate of claim 78, wherein said polymer molecule is a peptide comprising a promiscuous T helper cell epitope.

80. An immunizing composition, comprising an immunizing effective amount of the peptide of claim 70 or a conjugate thereof, and a pharmaceutically acceptable carrier, excipient, diluent, adjuvant, or auxiliary agent.

81. A method for inducing an immune response to amylin and amylin fibrils, comprising administering to a human subject in need thereof either the immunizing composition of claim 80 or

an immunizing composition comprising amylin from a mammalian species which does not form amylin fibrils.

82. The method of claim 81, wherein the mammalian species is mouse.

83. A method of reducing amyloidosis comprising administering the immunizing composition according to claim 80 to a subject in need thereof, thereby reducing amyloidosis.

84. A molecule which includes the antigen-binding portion of an antibody raised against the peptide of claim 70.

85. The molecule of claim 84 which is selected from the group consisting of monoclonal antibody, single chain antibody, and humanized antibody.

86. A pharmaceutical composition, comprising the molecule of claim 84 and a pharmaceutically acceptable carrier, diluent, excipient or auxiliary agent.

87. A method for reducing the formation of amylin fibril, comprising administering the molecule of claim 84 to a human subject in need thereof.

88. A method of reducing amyloidosis comprising administering the pharmaceutical composition according to claim 86 to a subject in need thereof, thereby reducing amyloidosis.

89. An isolated polypeptide or peptide selected from the group consisting of:

(A) a polypeptide of SEQ ID NO:55 in which one or more of three sets of valine residues, (i) residues 37 and 40, (ii) residues 48, 49, and 52, and (iii) residues 70, 71, and 74, is substituted with all Glu, all Asp, all Pro, all Lys, all Gly, or all Ser residues;

(B) a peptide consisting of a 30 to 36 residue segment, corresponding to residues 1-30 to 1-36 of SEQ ID NO:54, alone or joined at its N-terminus and/or C-terminus to a polylysine or polyaspartate segment of 4-10 residues in length; and

(C) a peptide consisting of a 30 to 66 residue segment, corresponding to residues 75-140 to 111-140 of SEQ ID NO:54, alone or joined at its N-terminus and/or C-terminus to a polylysine or polyaspartate segment of 4-10 residues in length.

90. The isolated polypeptide or peptide of claim 89, wherein all residues are D-amino acid residues.

91. The isolated polypeptide or peptide of claim 89, which is a polypeptide of SEQ ID NO:55 in which one or more of three sets of valine residues, (i) residues 37 and 40, (ii) residues 48, 49, and 52, and (iii) residues 70, 71, and 74, is substituted with all Glu, all Asp, all Pro, all Lys, all Gly, or all Ser residues.

92. The isolated polypeptide or peptide of claim 89, which is a peptide consisting of a 30 to 36 residue segment, corresponding to residues 1-30 to 1-36 of SEQ ID NO:54, alone or

joined at its N-terminus and/or C-terminus to a polylysine or polyaspartate segment of 4-10 residues in length.

93. The isolated polypeptide or peptide of claim 89, which is a peptide consisting of a 30 to 66 residue segment, corresponding to residues 75-140 to 111-140 of SEQ ID NO:54, alone or joined at its N-terminus and/or C-terminus to a polylysine or polyaspartate segment of 4-10 residues in length.

94. A conjugate of the polypeptide or peptide of claim 89 cross-linked to a polymer molecule.

95. The conjugate of claim 94, wherein said polymer molecule is a peptide comprising a promiscuous T helper cell epitope.

96. An immunizing composition, comprising an immunizing effective amount of the polypeptide or peptide of claim 89 or a conjugate thereof, and a pharmaceutically acceptable carrier, excipient, diluent, adjuvant, or auxiliary agent.

97. A method for inducing an immune response to α-synuclein and Lewy bodies, comprising administering to a human subject in need thereof either the immunizing composition of claim 96 or an α-synuclein protein from a mammalian species that does not form filament or fibril deposits of α-synuclein.

98. A method of reducing amyloidosis, comprising administering the immunizing composition according to claim 96 to a subject in need thereof, thereby reducing amyloidosis.

99. A molecule which includes the antigen-binding portion of an antibody raised against the polypeptide or peptide of claim 89.

100. The molecule of claim 99 which is selected from the group consisting of monoclonal antibody, single chain antibody, and humanized antibody.

101. A pharmaceutical composition, comprising the molecule of claim 98 and a pharmaceutically acceptable carrier, diluent, excipient or auxiliary agent.

102. A method for reducing the formation of Lewy bodies, comprising administering the molecule of claim 99 to a human subject in need thereof.

103. A method of reducing amyloidosis, comprising administering the pharmaceutical composition according to claim 101, thereby reducing amyloidosis.

104. An polypeptide or peptide, comprising a polyglutamine segment of 30-200 glutamine residues in length joined at its N-terminus and/or C-terminus to a polylysine or polyaspartate segment of 4-10 residues in length.

105. The isolated polypeptide or peptide of claim 104, wherein all residues are D-amino acid residues.

106. A conjugate of the polypeptide or peptide of claim 104 cross-linked to a polymer molecule.

107. The conjugate of claim 106, wherein said polymer molecule is a peptide comprising a promiscuous T helper cell epitope.

108. An immunizing composition, comprising an immunizing effective amount of the polypeptide or peptide of claim 104 or a conjugate thereof, and a pharmaceutically acceptable carrier, excipient, diluent, adjuvant, or auxiliary agent.

109. A method for inducing an immune response to a protein with polyglutamine repeats that is associated with neurodegenerative movement disorders, comprising administering to a human subject in need thereof the immunizing composition of claim 108.

110. A method of reducing amyloidosis, comprising administering the immunizing composition according to claim 108 to a subject in need thereof, thereby reducing amyloidosis.

111. A molecule which includes the antigen-binding portion of an antibody raised against the polypeptide or peptide of claim 104.

112. The molecule of claim 111 which is selected from the group consisting of monoclonal antibody, single chain antibody, and humanized antibody.

113. A pharmaceutical composition, comprising the molecule of claim 111 and a pharmaceutically acceptable carrier, diluent, excipient or auxiliary agent.

114. A method for reducing formation of protein aggregates containing polyglutamine repeats, comprising administering the molecule of claim 111 to a human subject in need thereof.

115. A method of reducing amyloidosis, comprising administering the pharmaceutical composition according to claim 113 to a subject in need thereof, thereby reducing amyloidosis.

F I G. 1
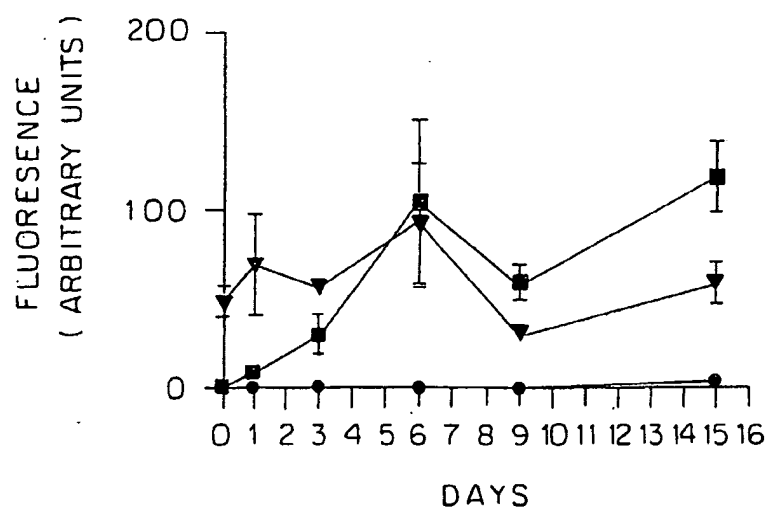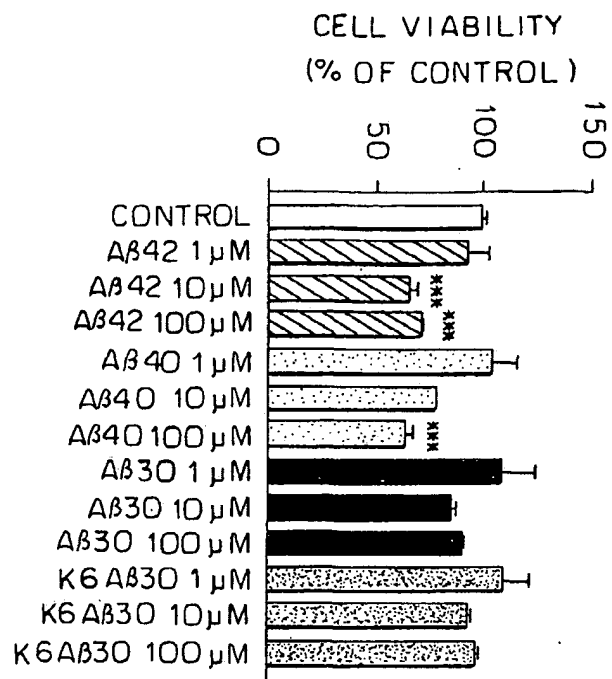
FIG. 2A
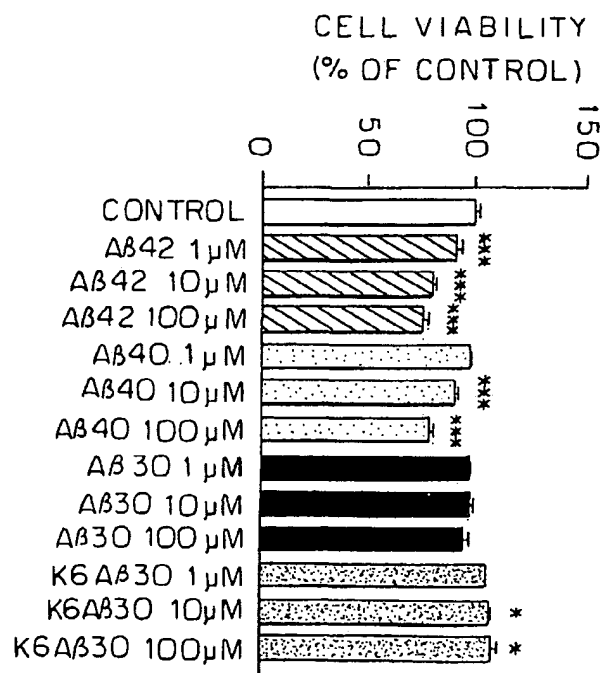
FIG. 2B

FIG.3A

FIG.3B

FIG.3C

FIG.3D

FIG. 4A

FIG. 4B

FIG. 4C

ELISA FOR SOLUBLE Aβ

FIG. 5

2 Days

FIG. 6A

6 Days

FIG. 6B

FIG. 7

10-fold Dilution of 139A Brain Homogenate Inoculum

FIG. 8A

1000-fold Dilution of 139A Brain Homogenate Inoculum

FIG. 8B

FIG.9
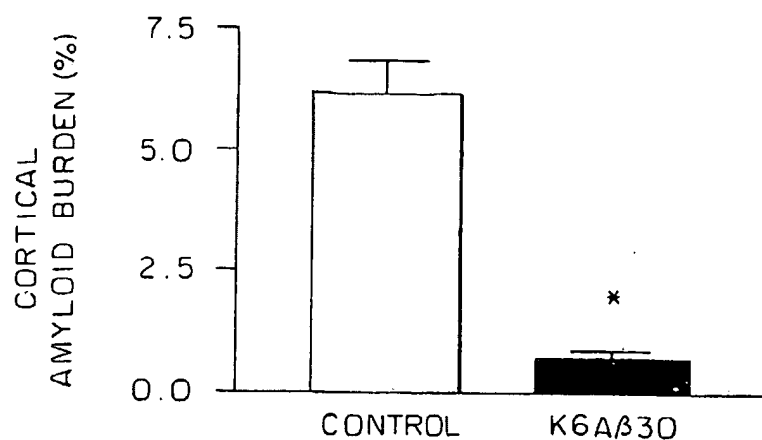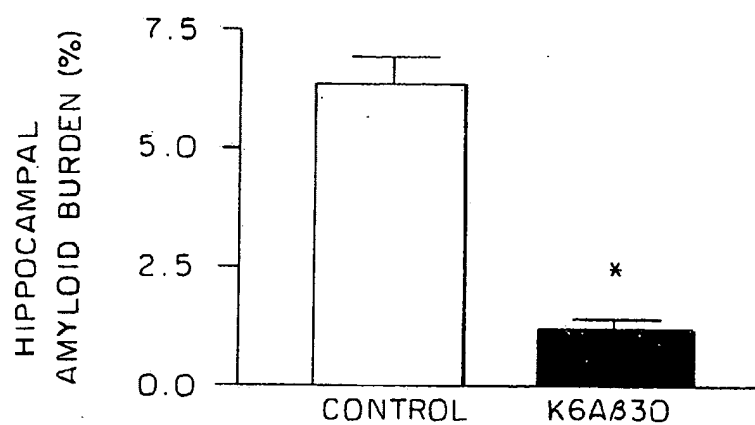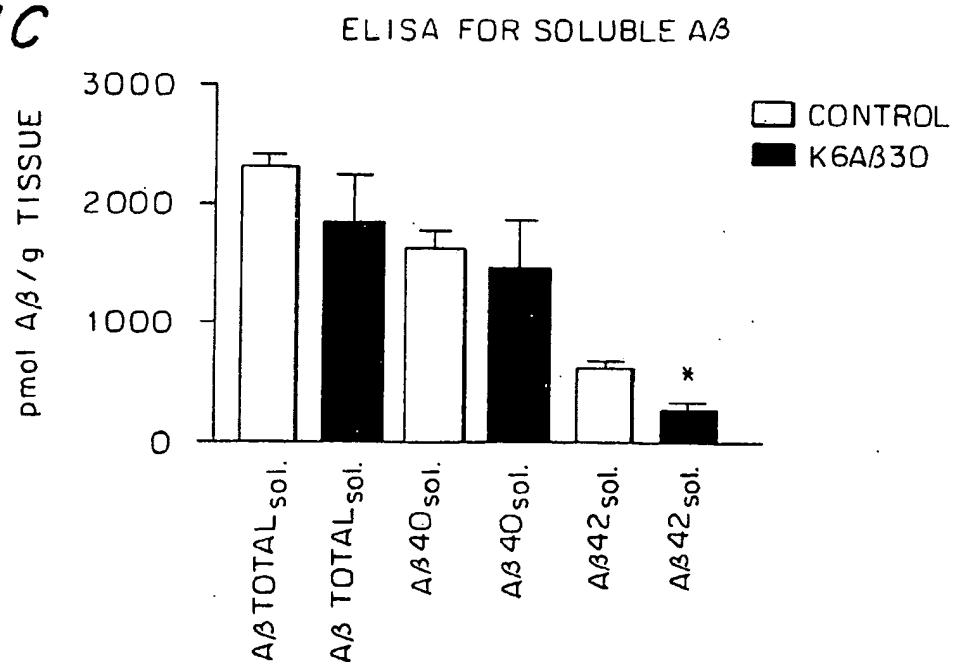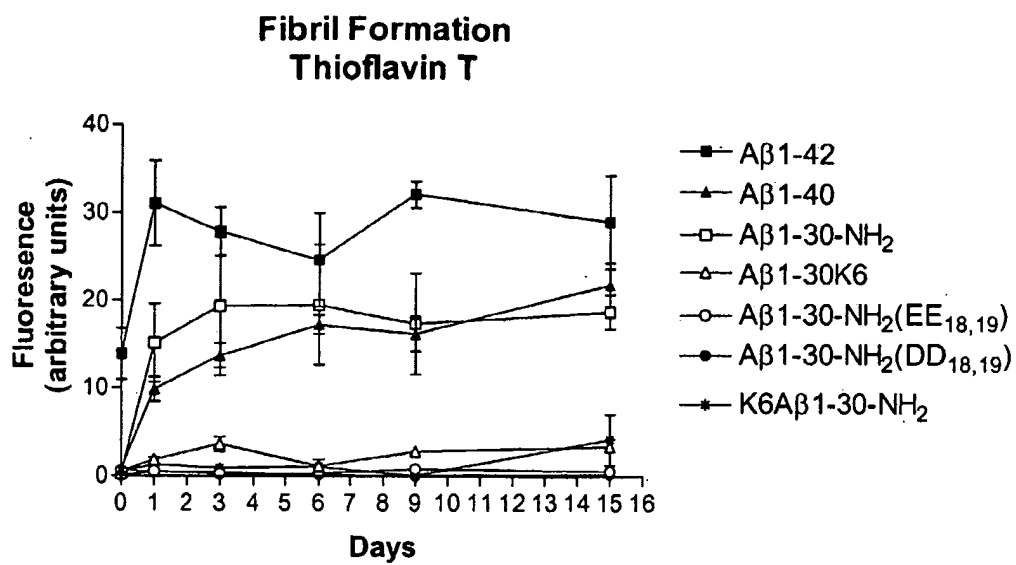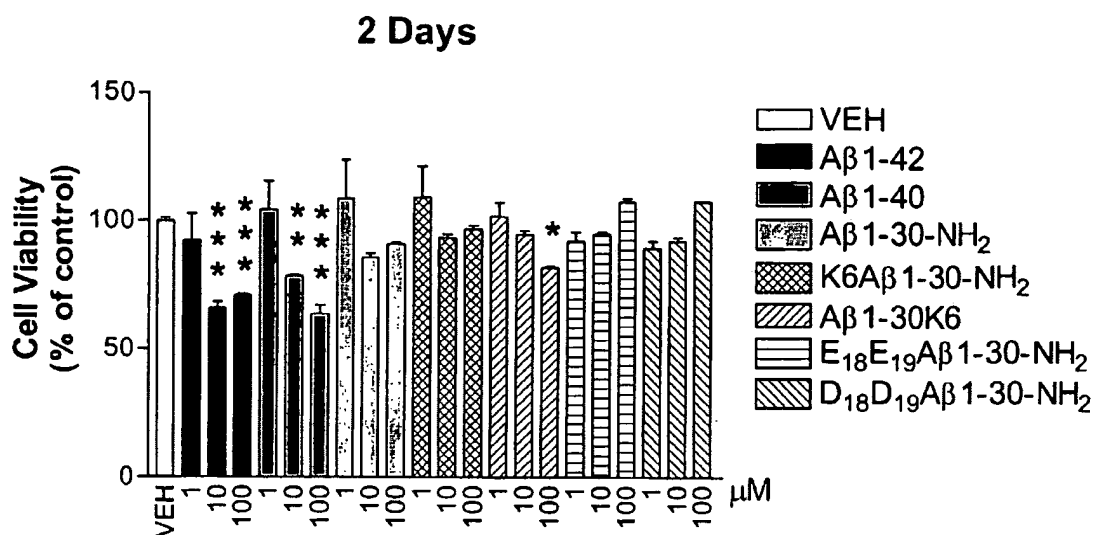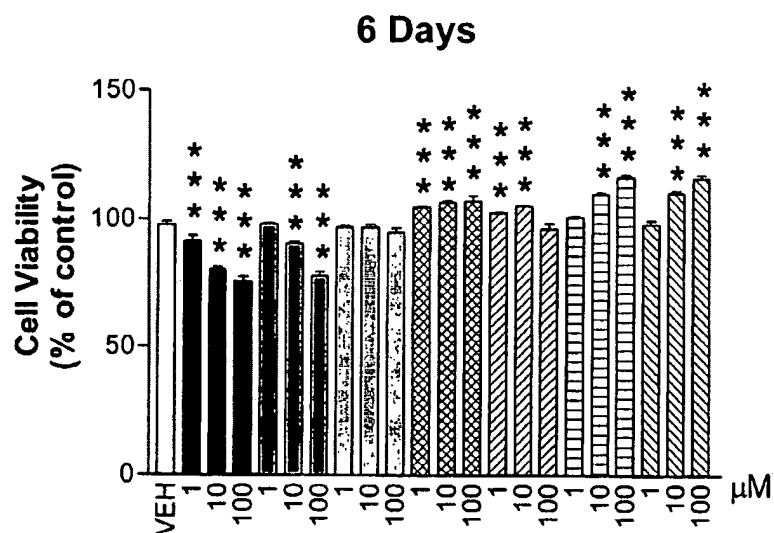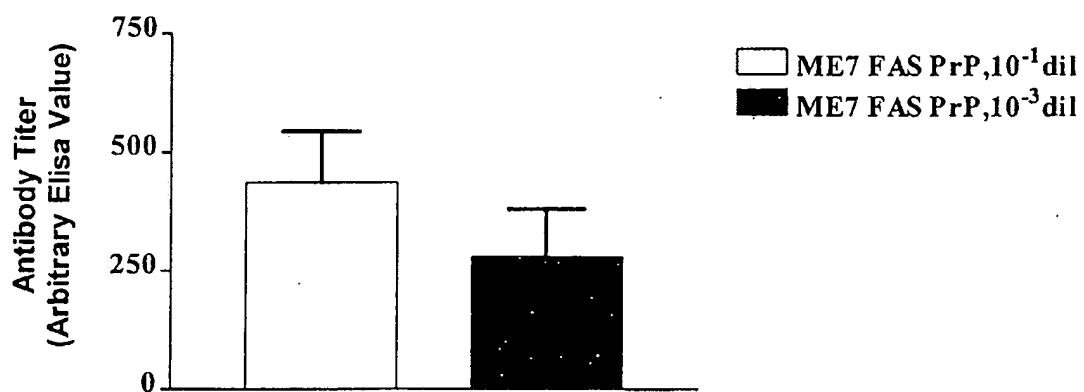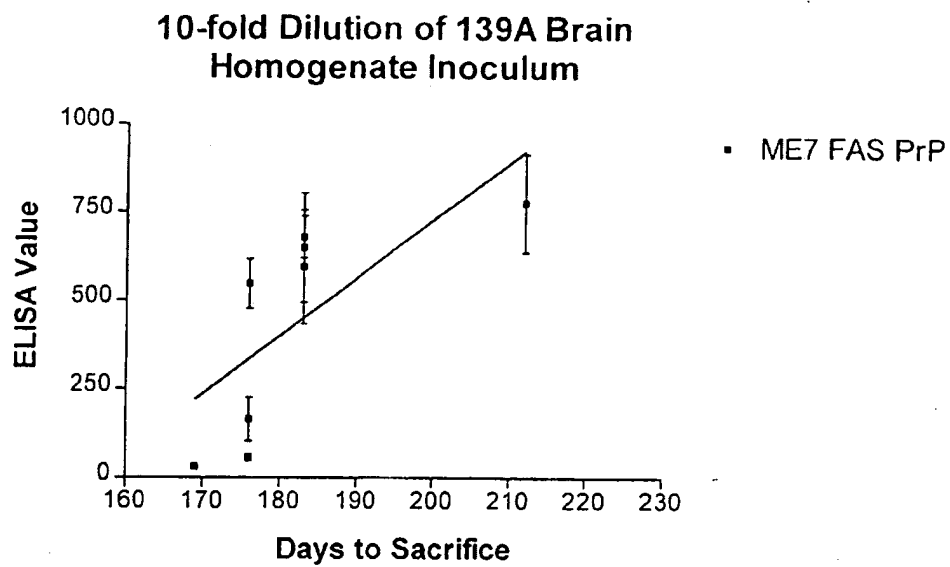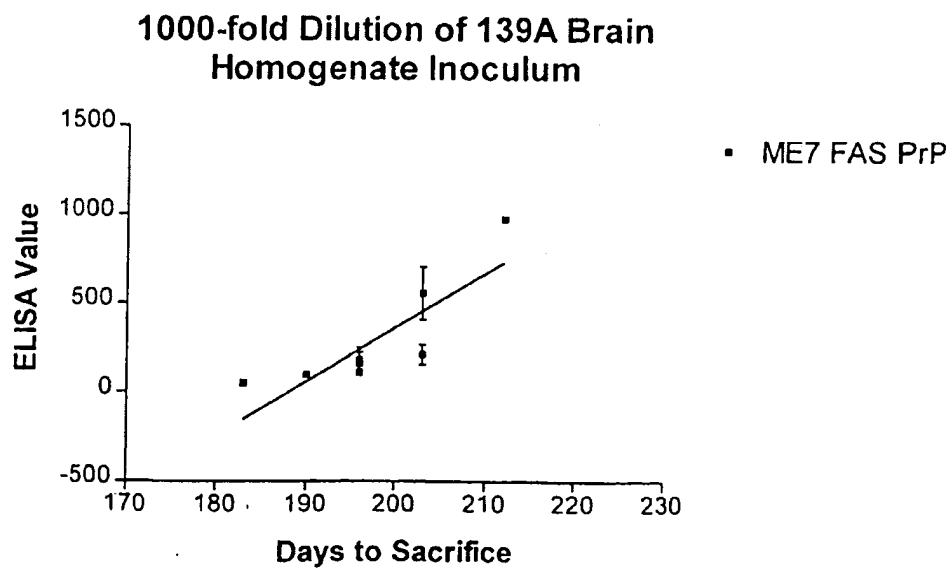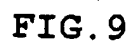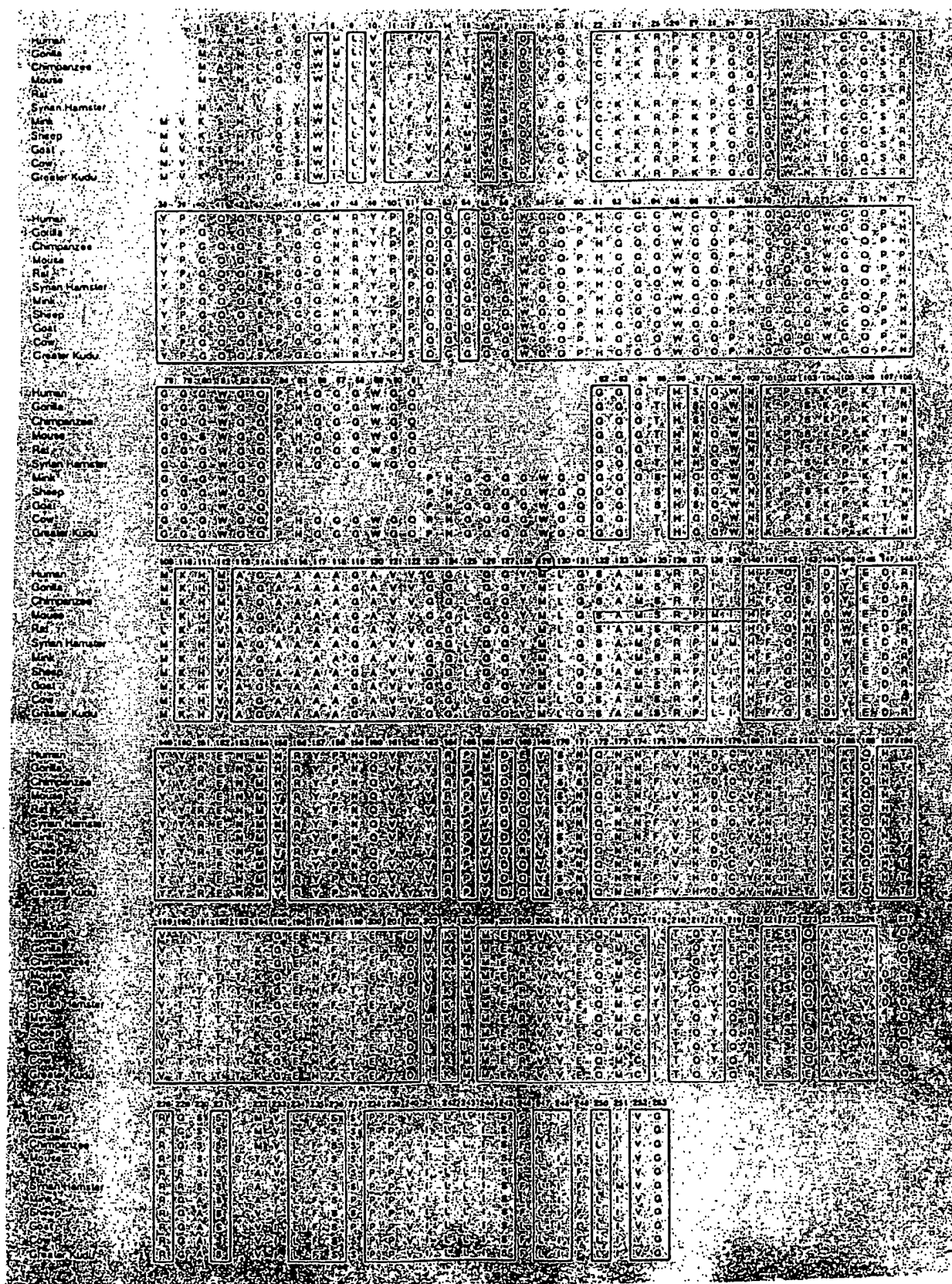
FIG.10

Wells coated with K6Aβ30

FIG. 11A

Wells coated with Aβ42

FIG. 11B

FIG. 11C

Wells coated with Aβ42

FIG. 12A

Wells coated with K6Aβ30

FIG. 12B

Wells coated with Aβ40

FIG. 12C

NORTH
SOUTH

A.

REINFORCER
REINFORCER

GOAL BOX
GOAL BOX

B.
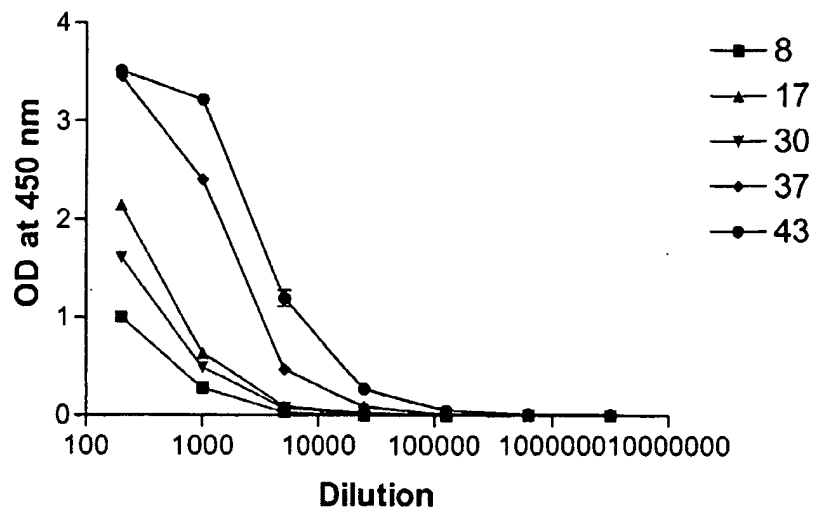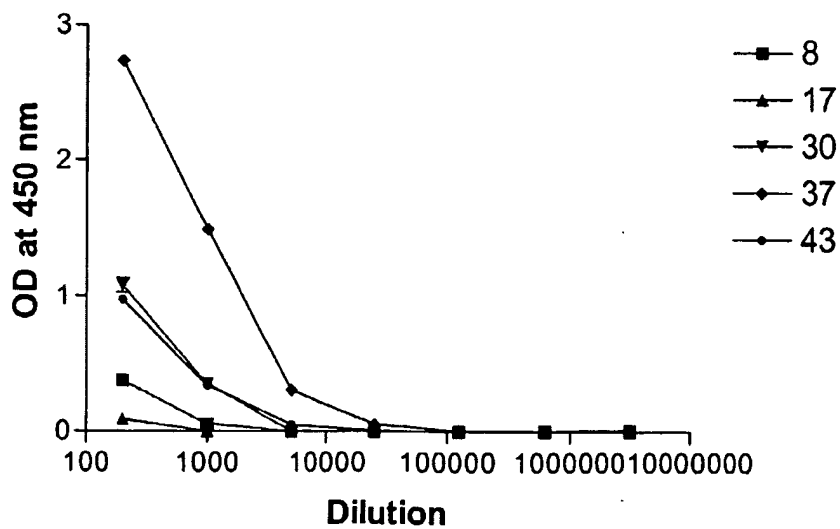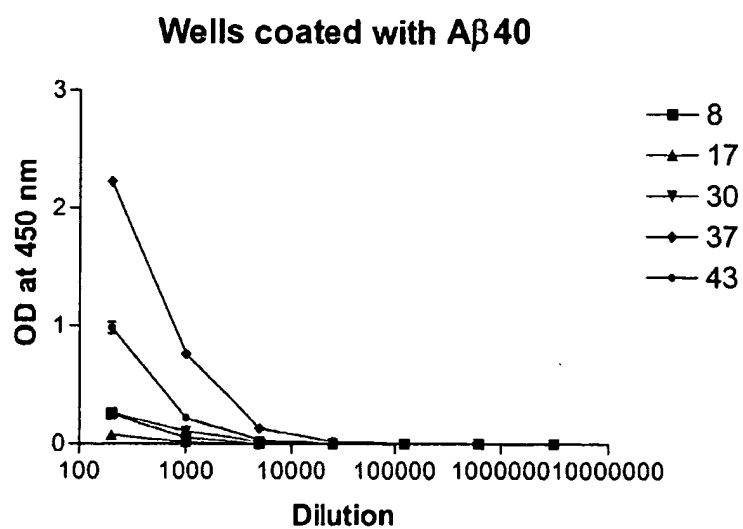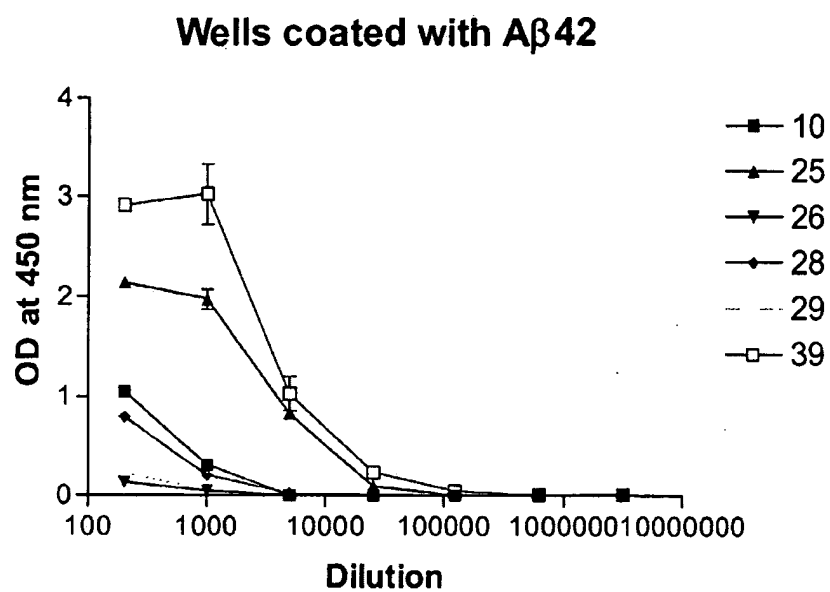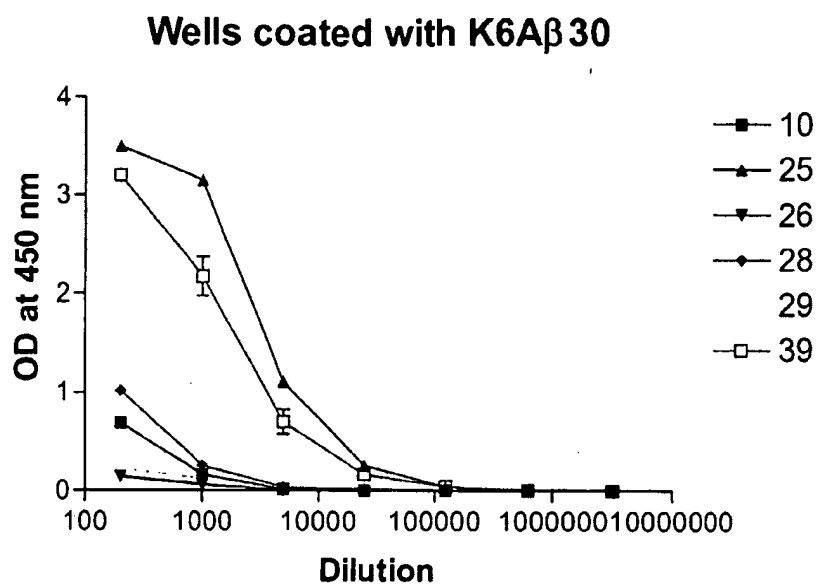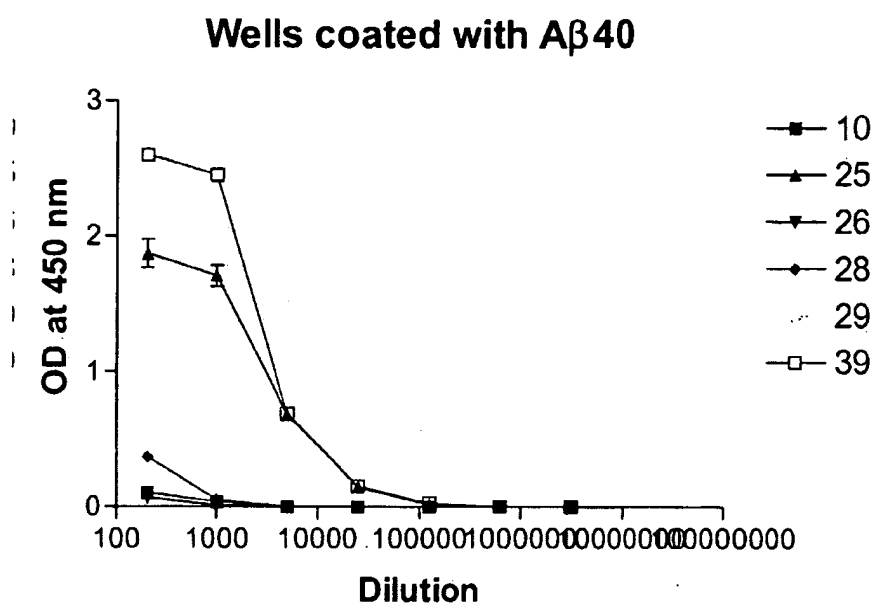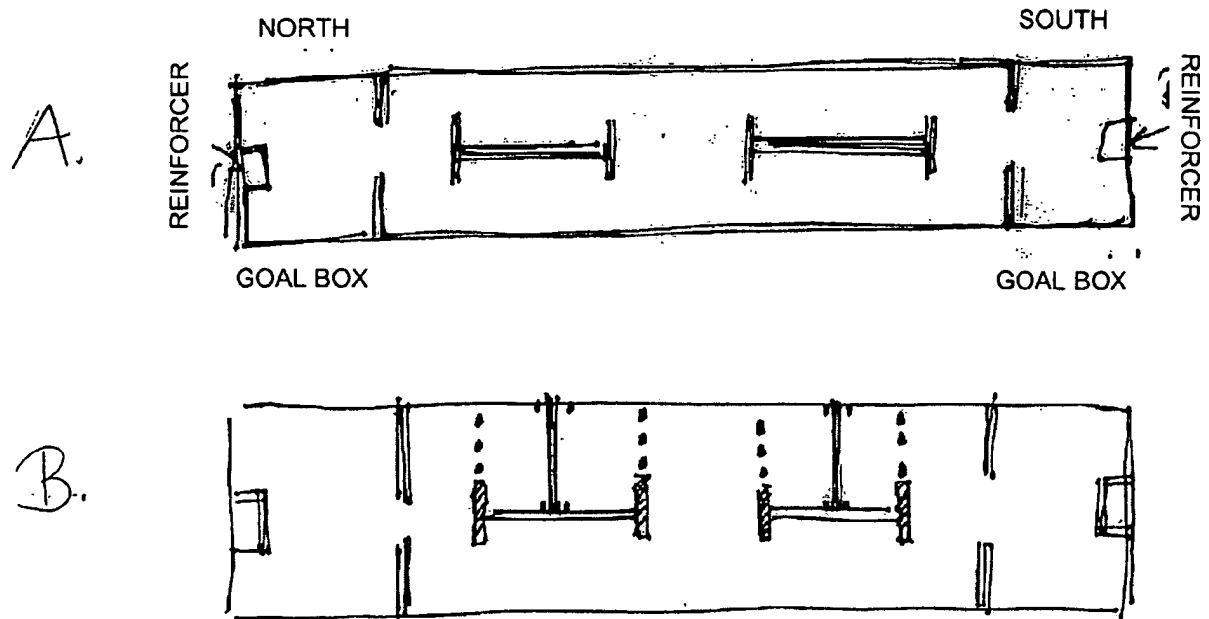
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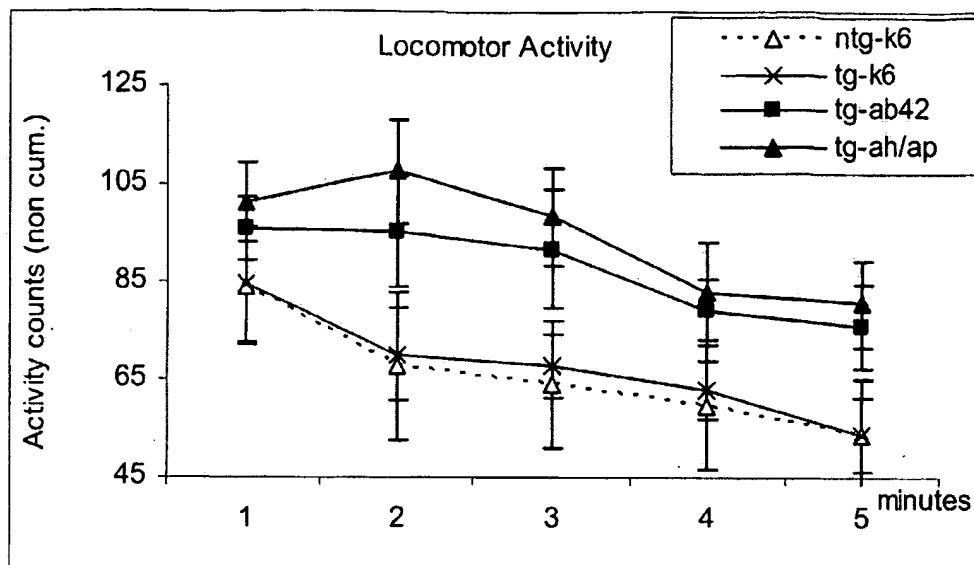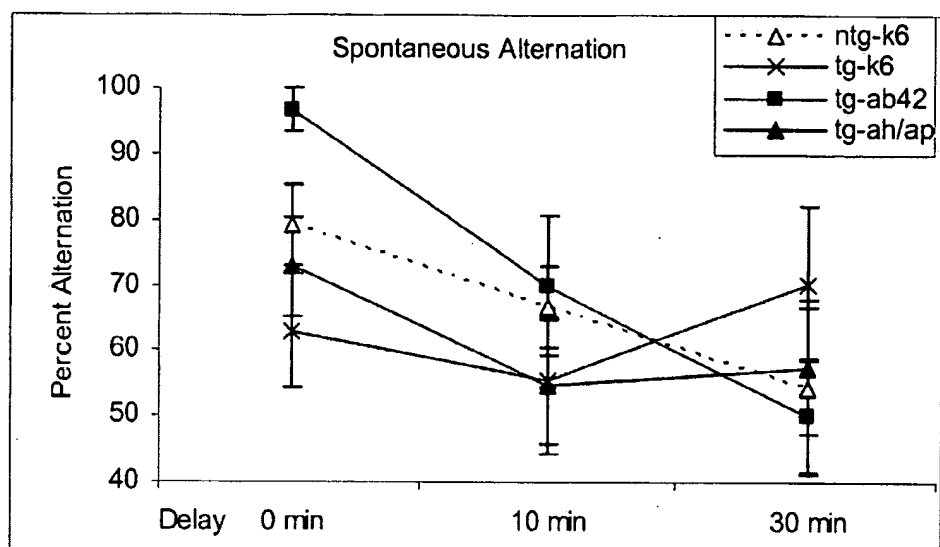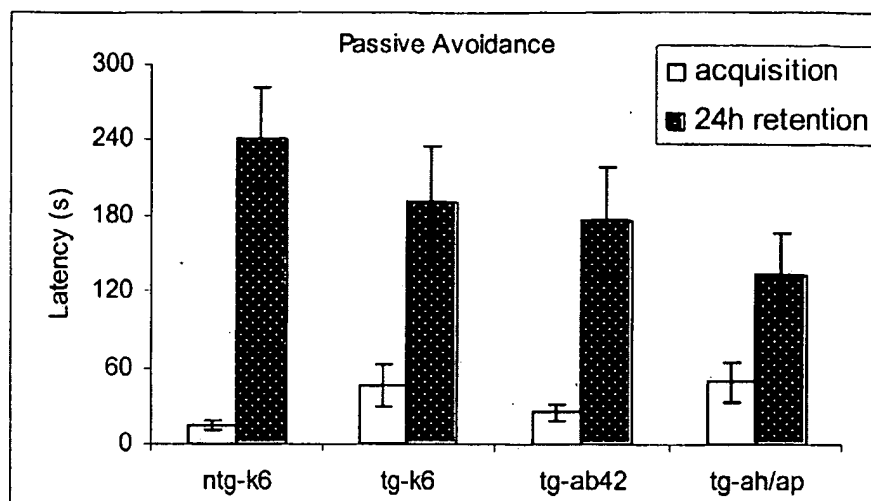
FIG. 14A

FIG. 14B

FIG. 14C
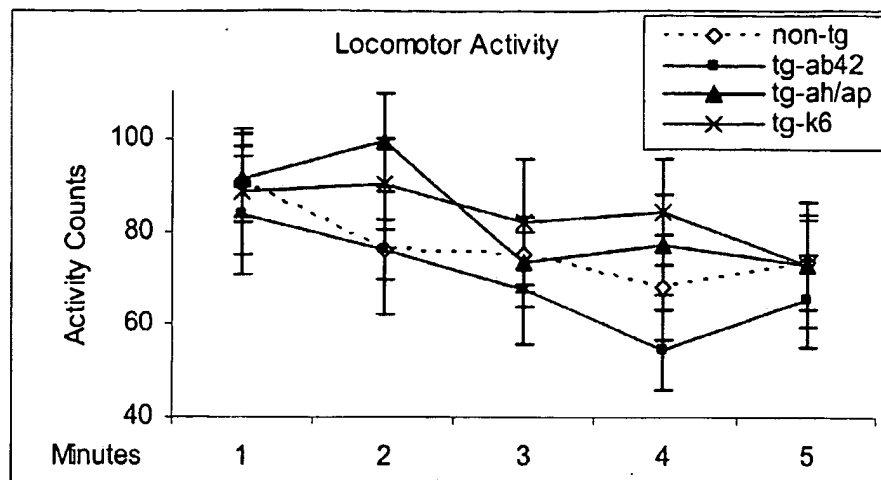
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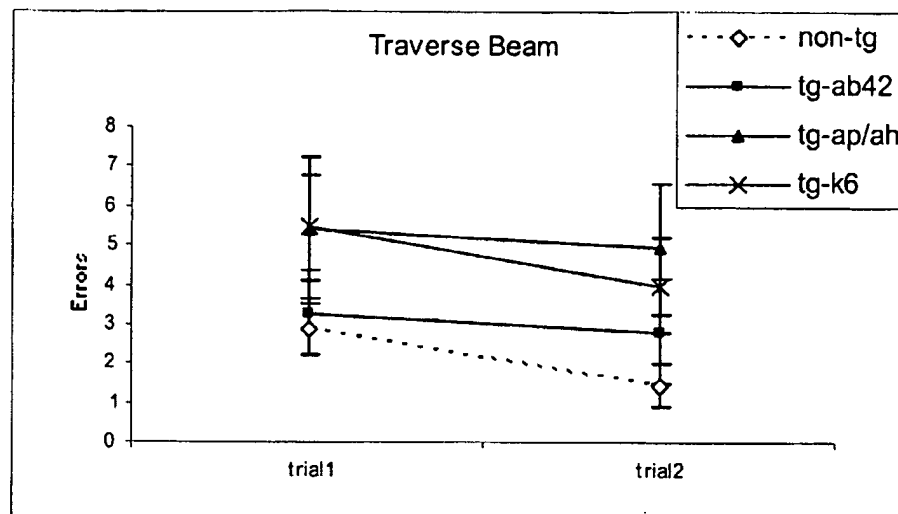
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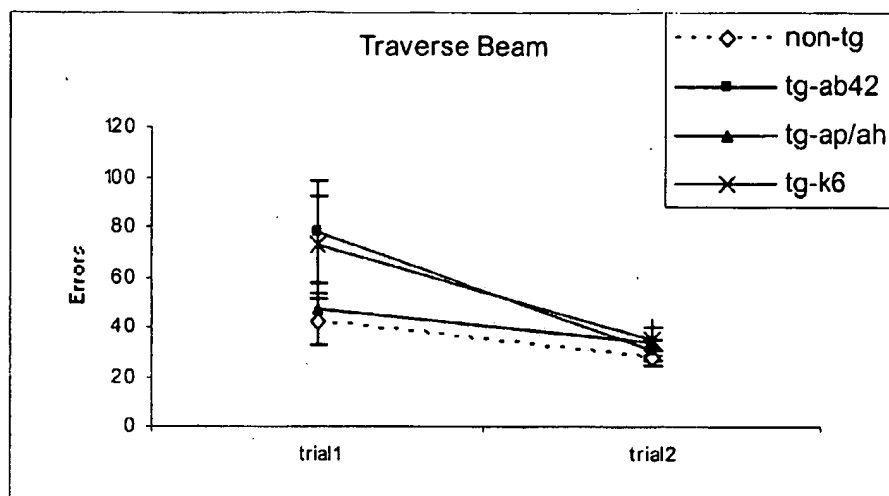
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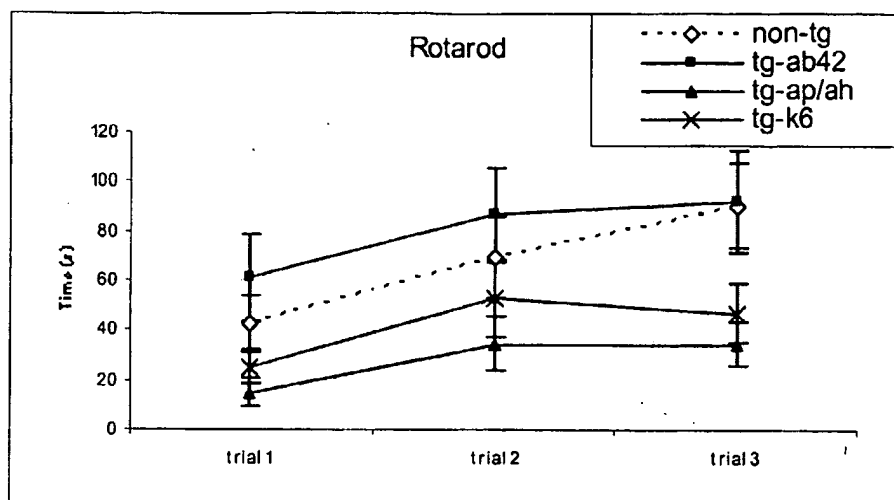
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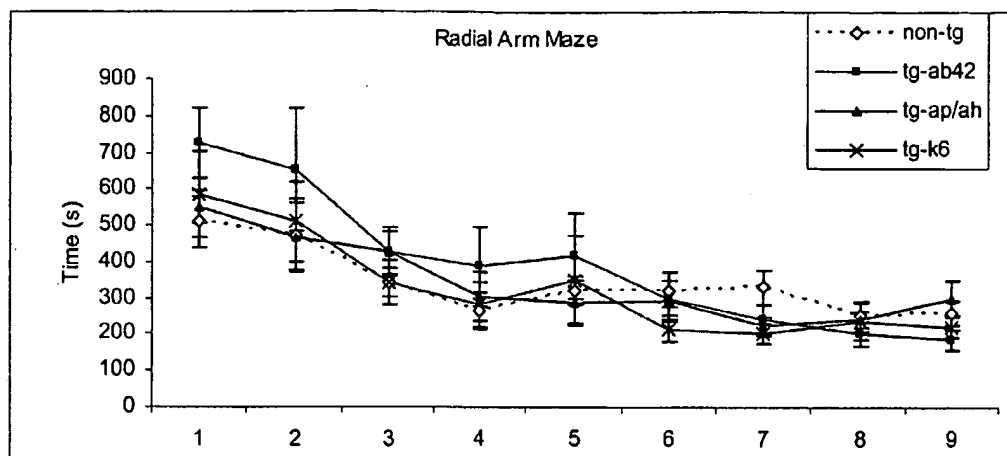
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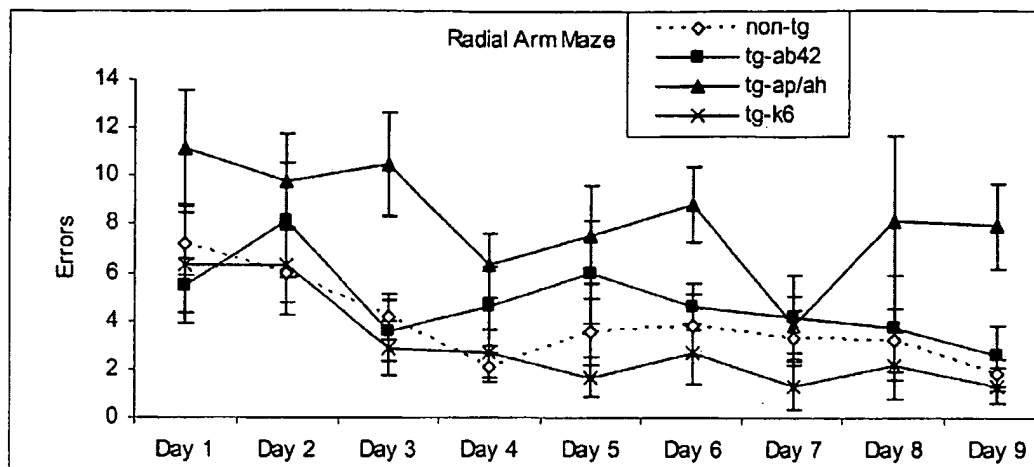
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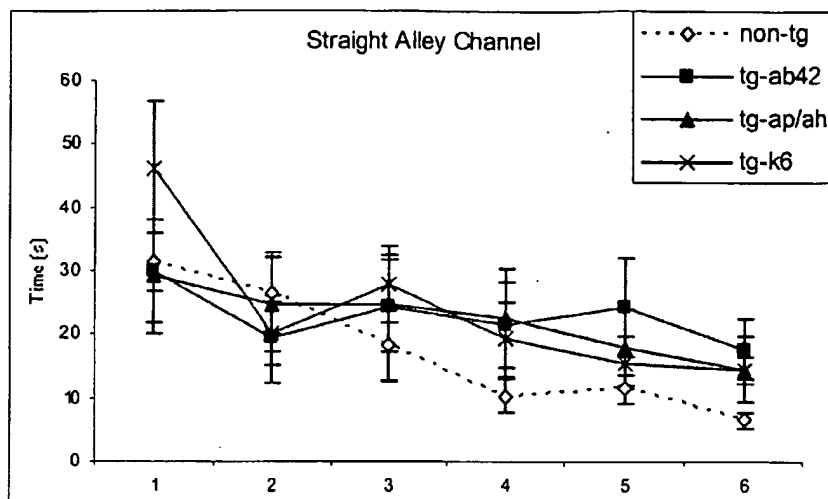
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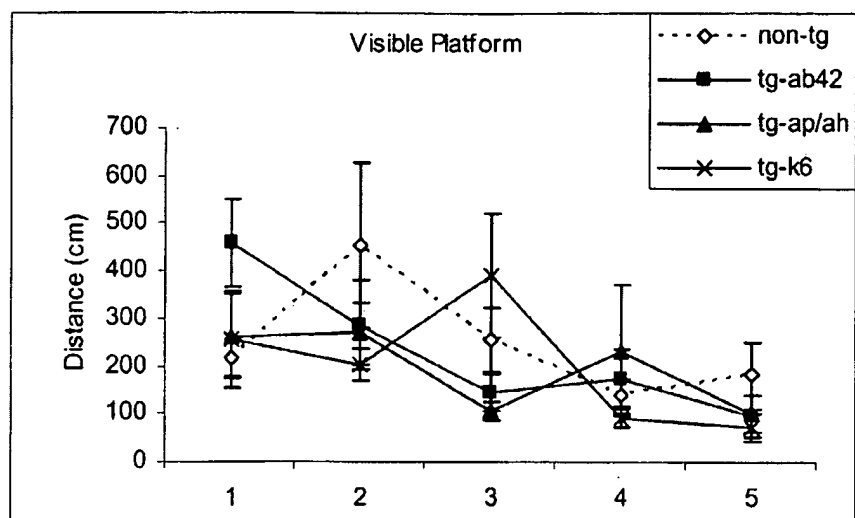
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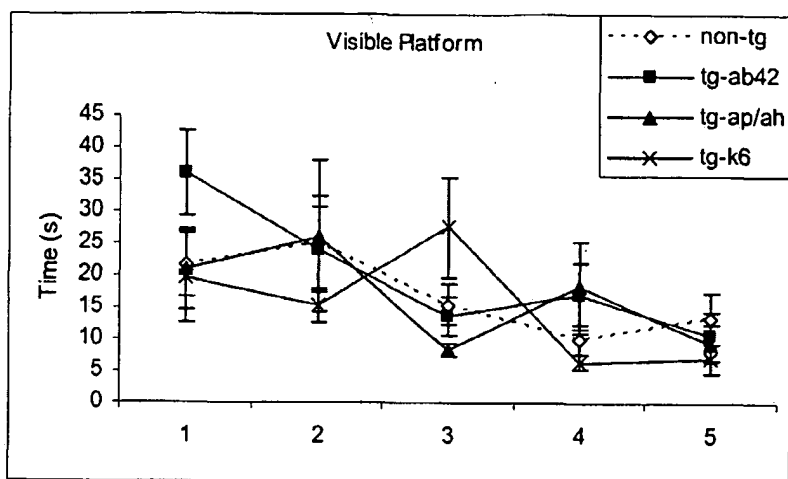
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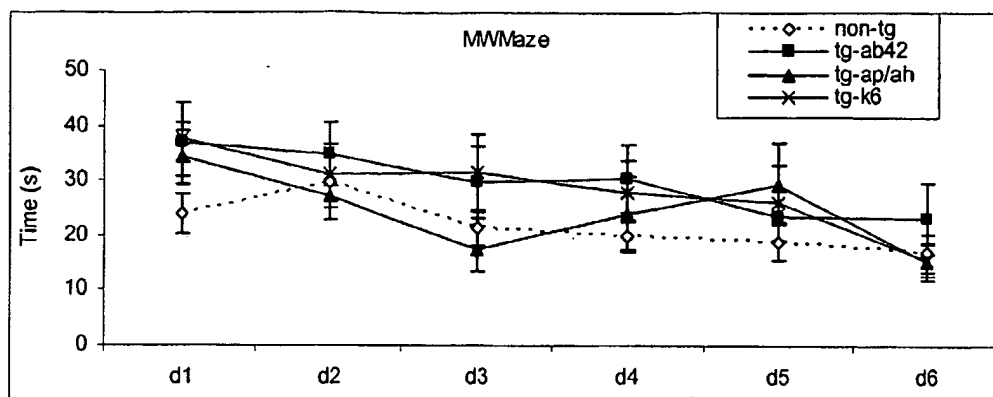
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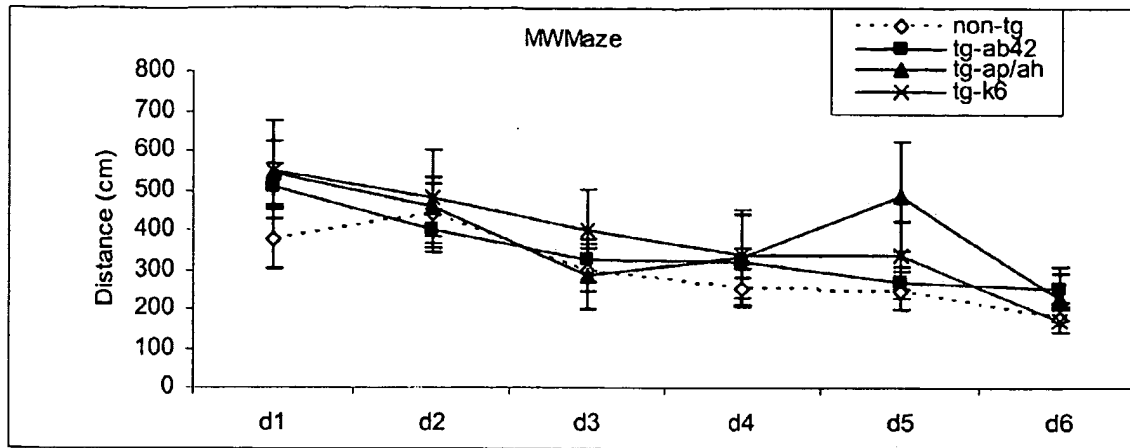
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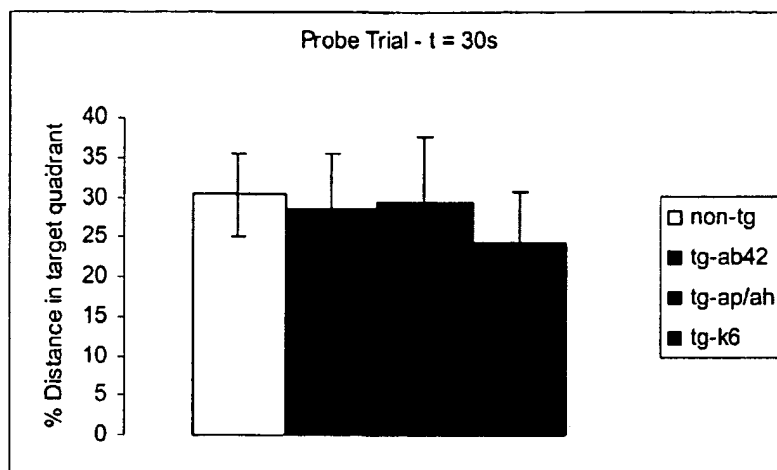
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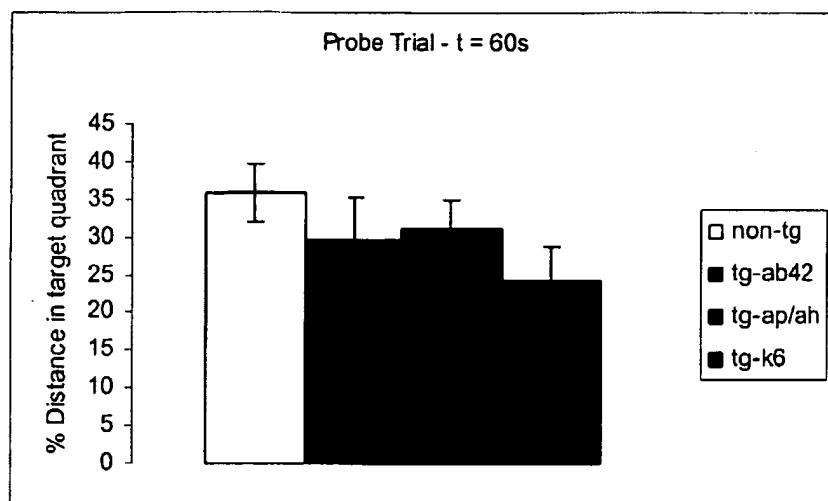
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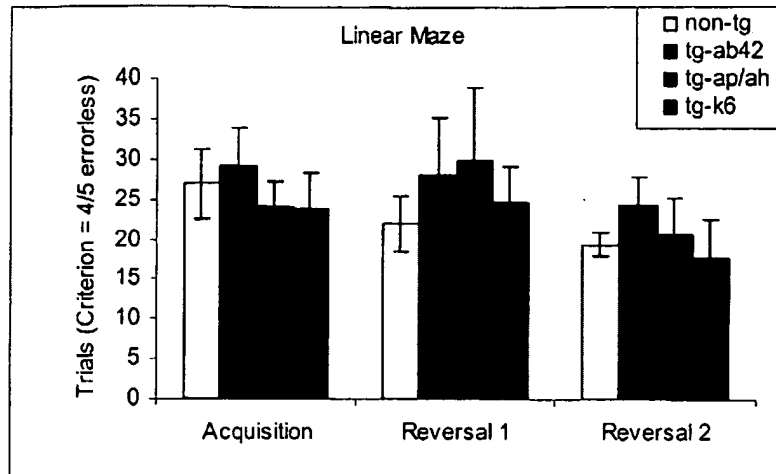
FIG. 15N

SEQUENCE LISTING

<110> NYU MEDICAL CENTER
      FRANGIONE, Blas
      WISNIEWSKI, Thomas
      SIGURDSSON, Einar

<120> SYNTHETIC IMMUNOGENIC BUT NON-DEPOSIT- FORMING POLYPEPTIDES AND
PEPTIDES HOMOLOGOUS TO AMYLOID BETA, PRION PROTEIN, AMYLIN, ALPHA-SYNUCLEIN,
OR POLYGLUTAMINE REPEATS FOR INDUCTION OF AN IMMUNE RESPONSE THERETO

<130> 5986/2K434WOO

<150> US 60/331,801
<151> 2001-11-21

<160> 55

<170> PatentIn version 3.1

<210> 1
<211> 30
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic

<400> 1

Asp Ala Glu Phe Arg His Asp Ser Gly Tyr Glu Val His His Gln Lys
1   5   10   15

Leu Val Phe Phe Ala Glu Asp Val Gly Ser Asn Lys Gly Ala
            20   25   30

<210> 2
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<220>
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<223> Amino acid residues 7-10 either are present, together as all Lys
      or all Asp, or are all absent. When residues 7-10 are present
      then any one or all of residues 1-6 can either be absent or present as
      Lys or Asp to form, in combination with residues 7-10, a N-terminal
      polylysine or polyaspartate segment of 4 to 10 residues in length.

<220>
<221> misc_feature
<222> (27)..(31)
<223> Amino acid residues 27-31 are LeuValPhePheAla in which at least one
      of residues 27-31 are substituted with Lys, Asp, or Glu. The C-

terminal Ala residue may be amidated.

<400> 2

Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Asp Ala Glu Phe Arg His
1   5   10   15

Asp Ser Gly Tyr Glu Val His His Gln Lys Xaa Xaa Xaa Xaa Xaa Glu
            20   25   30

Asp Val Gly Ser Asn Lys Gly Ala
            35   40

<210> 3
<211> 70
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<220>
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<223> Amino acid residues 7-10 are all Lys or all Asp, or are all absent.
      When residues 7-10 are present, then any one or all of amino acid
      residues 1-6 can either be absent or present as Lys or Asp to form, in
      combination with residues 7-10, a N- terminal polylysine or
      polyaspartate segment of 4 to 10 residues in length.

<220>
<221> misc_feature
<222> (27)..(31)
<223> Amino acid residues 27-31 and 57-61 are the same and are
      LeuValPhePheAla in which at least one of residues 27-31 and the same
      at least one residues of residues 57-61 are substituted with Lys, Asp,
      or Glu. The C-terminal Ala residue may be amidated.

<220>
<221> misc_feature
<222> (57)..(61)
<223> Amino acid residues 27-31 and 57-61 are the same and are
      LeuValPhePheAla in which at least one of residues 27-31 and the same
      at least one residues of residues 57-61 are substituted with Lys, Asp,
      or Glu. The C-terminal Ala residue may be amidated.

<400> 3

Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Asp Ala Glu Phe Arg His
1   5   10   15

Asp Ser Gly Tyr Glu Val His His Gln Lys Xaa Xaa Xaa Xaa Xaa Glu
            20   25   30

```
Asp Val Gly Ser Asn Lys Gly Ala Asp Ala Glu Phe Arg His Asp Ser
        35                  40                  45


Gly Tyr Glu Val His His Gln Lys Xaa Xaa Xaa Xaa Xaa Glu Asp Val
        50                  55                  60


Gly Ser Asn Lys Gly Ala
65                  70
```

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<210>  4
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<212>  PRT
<213>  Artificial Sequence

<220>
<223>  Synthetic

<220>
<221>  misc_feature
<222>  (31)..(40)
<223>  Amino acid residues 31-34 are all Lys or all Asp or are all absent.
       When all residues 31-34 are present, then any one or all of residues
       35-40 can either be absent or present as Lys or Asp to form, in
       combination with residues 31-34, a C-terminal polylysine or
       polyaspartate segment of 4 to 10 residues in length.


<220>
<221>  misc_feature
<222>  (17)..(21)
<223>  Amino acid residues 17-21 are LeuValPhePheAla in which at least one
       of residues 17-21 is substituted with Lys, Asp, or Glu.


<400>  4

Asp Ala Glu Phe Arg His Asp Ser Gly Tyr Glu Val His His Gln Lys
1               5               10                  15


Xaa Xaa Xaa Xaa Xaa Glu Asp Val Gly Ser Asn Lys Gly Ala Xaa Xaa
            20                  25                  30


Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa
        35                  40
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<210>  5
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<213>  Artificial Sequence

<220>
<223>  Synthetic
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<220>
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       When all residues 61-64 are present, then any one or all of
       residues 65-70 can either be Lys or Asp to form, in combination
       with residues 61-64, a C-terminal polylysine or polyaspartate
       segment of 4 to 10 residues in length.


<220>
<221>  misc_feature
<222>  (17)..(21)
<223>  Amino acid residues 17-21 and 47-51 are the same and are
       LeuValPhePheAla in which at least one of residues 17-21 and the same
       at least one residues of 47-51 are the same at least one residues
       substituted with Lys, Asp, or Glu.


<220>
<221>  misc_feature
<222>  (47)..(51)
<223>  Amino acid residues 17-21 and 47-51 are the same and are
       LeuValPhePheAla in which at least one of residues 17-21 and the same
       at least one residues of 47-51 are the same at least one residues
       substituted with Lys, Asp, or Glu.


<400>  5

Asp Ala Glu Phe Arg His Asp Ser Gly Tyr Glu Val His His Gln Lys
1               5                   10                  15


Xaa Xaa Xaa Xaa Xaa Glu Asp Val Gly Ser Asn Lys Gly Ala Asp Ala
            20                  25                  30


Glu Phe Arg His Asp Ser Gly Tyr Glu Val His His Gln Lys Xaa Xaa
        35                  40                  45


Xaa Xaa Xaa Glu Asp Val Gly Ser Asn Lys Gly Ala Xaa Xaa Xaa Xaa
    50                  55                  60


Xaa Xaa Xaa Xaa Xaa Xaa
65                  70


<210>  6
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<212>  PRT
<213>  Artificial Sequence

<220>
<223>  Synthetic

<220>
<221>  misc_feature
<223>  C-terminal residue 36 may be amidated.
```

-4-

<400> 6

Lys Lys Lys Lys Lys Lys Asp Ala Glu Phe Arg His Asp Ser Gly Tyr
1   5   10   15

Glu Val His His Gln Lys Leu Val Phe Phe Ala Glu Asp Val Gly Ser
            20   25   30

Asn Lys Gly Ala
        35

<210> 7
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<213> Artificial Sequence

<220>
<223> Synthetic

<220>
<221> misc_feature
<222> (1)..(10)
<223> Amino acid residues 1-6 can either be absent or present as Lys or
      Asp to form, in combination with residues 7-10, a N-terminal
      polylysine or polyaspartate segment of 4 to10 residues in length.

<220>
<221> misc_feature
<223> The C-terminal Ala residue may be amidated.

<400> 7

Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Asp Ala Glu Phe Arg His
1   5   10   15

Asp Ser Gly Tyr Glu Val His His Gln Lys Leu Val Phe Phe Ala Glu
            20   25   30

Asp Val Gly Ser Asn Lys Gly Ala
        35   40

<210> 8
<211> 40
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic

<220>
<221> misc_feature

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<222>   (31)..(40)
<223>   Amino acid residues 35-40 can either be absent or present as Lys
        or Asp to form, in combination with residues 31-34, a C-terminal
        polylysine or polyaspartate segment of 4-10 residues in length.


<400>   8

Asp Ala Glu Phe Arg His Asp Ser Gly Tyr Glu Val His His Gln Lys
1               5                   10                  15


Leu Val Phe Phe Ala Glu Asp Val Gly Ser Asn Lys Gly Ala Xaa Xaa
            20                  25                  30


Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa
            35                  40


<210>   9
<211>   50
<212>   PRT
<213>   Artificial Sequence

<220>
<223>   Synthetic

<220>
<221>   misc_feature
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<223>   Amino acid residues 7-10 are all Lys or all Asp.  Any one or all
        of residues 1-6 can either be absent or present as Lys or Asp to
        form, in combination with residues 7-10, a N-terminal polylysine
        or polyaspartate segment of 4 to 10 residues in length.


<220>
<221>   misc_feature
<222>   (27)..(31)
<223>   Amino acid residues 27-31 are LeuValPhePheAla in which at least one
        of residues 27-31 are substituted with Lys, Asp, or Glu.


<220>
<221>   misc_feature
<222>   (41)..(50)
<223>   Amino acid residues 41-44 are all Lys or all Asp.  Any one or all
        of residues 45-50 can be either absent or present as Lys or Asp
        to form, in combination with residues 41-44, a C-terminal
        polysine or polyaspartate segment of 4-10 residues.


<400>   9

Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Asp Ala Glu Phe Arg His
1               5                   10                  15


Asp Ser Gly Tyr Glu Val His His Gln Lys Xaa Xaa Xaa Xaa Xaa Glu
            20                  25                  30
```

```
Asp Val Gly Ser Asn Lys Gly Ala Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa
        35                  40                  45


Xaa Xaa
    50



<210> 10
<211> 80
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic

<220>
<221> misc_feature
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<223> Amino acid residues 7-10 are all Lys or all Asp.  Any one or all
      of amino acid residues 1-6 can either be absent or present as Lys
      or Asp to form, in combination with residues 7-10, a N-terminal
      polylysine or polyaspartate segment of 4 to 10 residues in length.


<220>
<221> misc_feature
<222> (27)..(31)
<223> Amino acid residues 27-31 and 57-61 are the same and are
      LeuValPhePheAla in which at least one of residues 27-31 and the same
      at least one residues of residues 57-61 are substituted with Lys, Asp,
      or Glu.


<220>
<221> misc_feature
<222> (57)..(61)
<223> Amino acid residues 27-31 and 57-61 are the same and are
      LeuValPhePheAla in which at least one of residues 27-31 and the same
      at least one residues of residues 57-61 are substituted with Lys, Asp,
      or Glu.


<220>
<221> misc_feature
<222> (71)..(80)
<223> Amino acid residues 71-74 are all Lys or all Asp.  Any one or all
      of residues 75-80 can either be absent or present as Lys or Asp
      to form, in combination with residues 71-74, a C-terminal
      polylysine or polyaspartate segment of 4 to 10 residues.


<400> 10

Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Asp Ala Glu Phe Arg His
1               5                   10                  15


Asp Ser Gly Tyr Glu Val His His Gln Lys Xaa Xaa Xaa Xaa Xaa Glu
```

                    20   25   30

Asp Val Gly Ser Asn Lys Gly Ala Asp Ala Glu Phe Arg His Asp Ser
        35   40   45

Gly Tyr Glu Val His His Gln Lys Xaa Xaa Xaa Xaa Xaa Glu Asp Val
        50   55   60

Gly Ser Asn Lys Gly Ala Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa
65   70   75   80

<210> 11
<211> 36
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic

<400> 11

Asp Ala Glu Phe Arg His Asp Ser Gly Tyr Glu Val His His Gln Lys
1   5   10   15

Leu Val Phe Phe Ala Glu Asp Val Gly Ser Asn Lys Gly Ala Lys Lys
            20   25   30

Lys Lys Lys Lys
        35

<210> 12
<211> 30
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic

<400> 12

Asp Ala Glu Phe Arg His Asp Ser Gly Tyr Glu Val His His Gln Lys
1   5   10   15

Leu Glu Glu Phe Ala Glu Asp Val Gly Ser Asn Lys Gly Ala
            20   25   30

<210> 13
<211> 30
<212> PRT
<213> Artificial Sequence

<220>

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<223>   Synthetic

<400>   13

Asp Ala Glu Phe Arg His Asp Ser Gly Tyr Glu Val His His Gln Lys
1               5                   10                  15


Leu Asp Asp Phe Ala Glu Asp Val Gly Ser Asn Lys Gly Ala
                20                  25                  30


<210>   14
<211>   5
<212>   PRT
<213>   Artificial Sequence

<220>
<223>   Synthetic

<400>   14

Leu Pro Phe Phe Asp
1               5


<210>   15
<211>   40
<212>   PRT
<213>   Artificial Sequence

<220>
<223>   Synthetic

<220>
<221>   misc_feature
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<223>   Amino acid residues 7-10 either are present, together as all Lys
        or all Asp, or are all absent.  When residues 7-10 are present then
        any one or all of residues 1-6 can either be absent or present
        as Lys or Asp to form, in combination with residues 7-10, a
        N-terminal polylysine or polyaspartate segment of 4 to10 residues in
        length.


<220>
<221>   misc_feature
<222>   (27)..(31)
<223>   Amino acid residues 27-31 are LeuValPhePheAla in which at least one
        of residues 27-31 are substituted with Pro, Gly, or Ser.  The C-
        terminal Ala residue may be amidated.


<400>   15

Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Asp Ala Glu Phe Arg His
1               5                   10                  15


Asp Ser Gly Tyr Glu Val His His Gln Lys Xaa Xaa Xaa Xaa Xaa Glu
                20                  25                  30
```

```
Asp Val Gly Ser Asn Lys Gly Ala
        35                  40


<210>  16
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<220>
<223>  Synthetic

<220>
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       When residues 7-10 are present, then any one or all of amino
       acid residues 1-6 can either be absent or present as Lys or Asp to
       form, in combination with residues 7-10, a N-terminal polylysine or
       polyaspartate segment of 4 to 10 residues in length.


<220>
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<223>  Amino acid residues 27-31 and 57-61 are the same and are
       LeuValPhePheAla in which at least one of residues 27-31 and the same
       at least one residues of residues 57-61 are substituted with Pro, Gly,
       or Ser.  The C-terminal Ala residue may be amidated.


<220>
<221>  misc_feature
<222>  (57)..(61)
<223>  Amino acid residues 27-31 and 57-61 are the same and are
       LeuValPhePheAla in which at least one of residues 27-31 and the same
       at least one residues of residues 57-61 are substituted with Pro, Gly,
       or Ser.  The C-terminal Ala residue may be amidated.


<400>  16

Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Asp Ala Glu Phe Arg His
1                   5                   10                  15


Asp Ser Gly Tyr Glu Val His His Gln Lys Xaa Xaa Xaa Xaa Xaa Glu
            20                  25                  30


Asp Val Gly Ser Asn Lys Gly Ala Asp Ala Glu Phe Arg His Asp Ser
            35                  40                  45


Gly Tyr Glu Val His His Gln Lys Xaa Xaa Xaa Xaa Xaa Glu Asp Val
    50                  55                  60


Gly Ser Asn Lys Gly Ala
```

65   70

<210> 17
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<213> Artificial Sequence

<220>
<223> Synthetic

<220>
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<222> (31)..(40)
<223> Amino acid residues 31-34 are all Lys or all Asp or are all
       absent. When all residues 31-34 are present, then any one or all of
       residues 35-40 can either be absent or present as Lys or Asp to
       form, in combination with residues 31-34, a C-terminal polylysine or
       polyaspartate segment of 4 to 10 residues in length.

<220>
<221> misc_feature
<222> (17)..(27)
<223> Amino acid residues 17-21 are LeuValPhePheAla in which at least one
       of residues 17-21 are substituted with Pro, Gly, or Ser.

<400> 17

Asp Ala Glu Phe Arg His Asp Ser Gly Tyr Glu Val His His Gln Lys
1   5   10   15

Xaa Xaa Xaa Xaa Xaa Glu Asp Val Gly Ser Asn Lys Gly Ala Xaa Xaa
            20   25   30

Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa
        35   40

<210> 18
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<213> Artificial Sequence

<220>
<223> Synthetic

<220>
<221> misc_feature
<222> (61)..(70)
<223> Amino acid residues 61-64 are all Lys or all Asp, or are all absent.
       When all residues 61-64 are present, then any one or all of
       residues 65-70 can either be Lys or Asp to form, in combination
       with residues 61-64, a C-terminal polylysine or polyaspartate segment
       of 4 to 10 residues in length.

<220>

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<221>  misc_feature
<222>  (17)..(21)
<223>  Amino acid residues 17-21 and 47-51 are the same and are
       LeuValPhePheAla in which at least one of residues 17-21 and the same
       at least one residues of 47-51 are the same at least one residues
       substituted with Pro, Gly, or Ser.


<220>
<221>  misc_feature
<222>  (47)..(51)
<223>  Amino acid residues 17-21 and 47-51 are the same and are
       LeuValPhePheAla in which at least one of residues 17-21 and the same
       at least one residues of 47-51 are the same at least one
       residues substituted with Pro, Gly, or Ser.


<400>  18

Asp Ala Glu Phe Arg His Asp Ser Gly Tyr Glu Val His His Gln Lys
1               5                   10                  15


Xaa Xaa Xaa Xaa Xaa Glu Asp Val Gly Ser Asn Lys Gly Ala Asp Ala
          20                  25                  30


Glu Phe Arg His Asp Ser Gly Tyr Glu Val His His Gln Lys Xaa Xaa
          35                  40                  45


Xaa Xaa Xaa Glu Asp Val Gly Ser Asn Lys Gly Ala Xaa Xaa Xaa Xaa
    50                  55                  60


Xaa Xaa Xaa Xaa Xaa Xaa
65                  70



<210>  19
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<220>
<223>  Synthetic

<220>
<221>  misc_feature
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<223>  Amino acid residues 7-10 are all Lys or all Asp.  Any one or all
       of residues 1-6 can either be absent or present as Lys or Asp to
       form, in combination with residues 7-10, a N-terminal polylysine
       or polyaspartate segment of 4-10 residues in length.


<220>
<221>  misc_feature
<222>  (27)..(31)
<223>  Amino acid residues 27-31 are LeuValPhePheAla in which at least one
       of residues 27-31 are substituted with Pro, Gly, or Ser.
```

```
<220>
<221>  misc_feature
<222>  (41)..(50)
<223>  Amino acid residues 41-44 are all Lys or all Asp.  Any one or all
       of residues 45-50 can be either absent or present as Lys or Asp
       to form, in combination with residues 41-44, a C-terminal polysine or
       polyaspartate segment of 4-10 residues.


<400>  19

Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Asp Ala Glu Phe Arg His
1               5                   10                  15


Asp Ser Gly Tyr Glu Val His His Gln Lys Xaa Xaa Xaa Xaa Xaa Glu
            20                  25                  30


Asp Val Gly Ser Asn Lys Gly Ala Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa
            35                  40                  45


Xaa Xaa
    50



<210>  20
<211>  80
<212>  PRT
<213>  Artificial Sequence

<220>
<223>  Synthetic

<220>
<221>  misc_feature
<222>  (1)..(10)
<223>  Amino acid residues 7-10 are all Lys or all Asp.  Any one or all
       of amino acid residues 1-6 can either be absent or present as Lys
       or Asp to form, in combination with residues 7-10, a N-terminal
       polylysine or polyaspartate segment of 4 to 10 residues in length.


<220>
<221>  misc_feature
<222>  (27)..(31)
<223>  Amino acid residues 27-31 and 57-61 are the same and are
       LeuValPhePheAla in which at least one of residues 27-31 and the same
       at least one residues of residues 57-61 are substituted with Lys, Asp,
       or Glu.


<220>
<221>  misc_feature
<222>  (57)..(61)
<223>  Amino acid residues 27-31 and 57-61 are the same and are
       LeuValPhePheAla in which at least one of residues 27-31 and the same
       at least one residues of residues 57-61 are substituted with Lys, Asp,
```

or Glu.

<220>
<221> misc_feature
<222> (71)..(80)
<223> Amino acid residues 71-74 are all Lys or all Asp. Any one or all
       of residues 75-80 can either be absent or present as Lys or Asp
       to form, in combination with residues 71-74, a C-terminal polylysine
       or polyaspartate segment of 4 to 10 residues.

<400> 20

Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Asp Ala Glu Phe Arg His
1   5   10   15

Asp Ser Gly Tyr Glu Val His His Gln Lys Xaa Xaa Xaa Xaa Xaa Glu
            20   25   30

Asp Val Gly Ser Asn Lys Gly Ala Asp Ala Glu Phe Arg His Asp Ser
            35   40   45

Gly Tyr Glu Val His His Gln Lys Xaa Xaa Xaa Xaa Xaa Glu Asp Val
    50   55   60

Gly Ser Asn Lys Gly Ala Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa
65   70   75   80

<210> 21
<211> 253
<212> PRT
<213> Homo sapiens

<400> 21

Met Ala Asn Leu Gly Cys Trp Met Leu Val Leu Phe Val Ala Thr Trp
1   5   10   15

Ser Asp Leu Gly Leu Cys Lys Lys Arg Pro Lys Pro Gly Gly Trp Asn
            20   25   30

Thr Gly Gly Ser Arg Tyr Pro Gly Gln Gly Ser Pro Gly Gly Asn Arg
            35   40   45

Tyr Pro Pro Gln Gly Gly Gly Gly Trp Gly Gln Pro His Gly Gly Gly
    50   55   60

Trp Gly Gln Pro His Gly Gly Gly Trp Gly Gln Pro His Gly Gly Gly
65   70   75   80

Trp Gly Gln Pro His Gly Gly Gly Trp Gly Gln Gly Gly Gly Thr His
                85   90   95

Ser Gln Trp Asn Lys Pro Ser Lys Pro Lys Thr Asn Met Lys His Met
            100   105   110

Ala Gly Ala Ala Ala Ala Gly Ala Val Val Gly Gly Leu Gly Gly Tyr
        115   120   125

Met Leu Gly Ser Ala Met Ser Arg Pro Ile Ile His Phe Gly Ser Asp
    130   135   140

Tyr Glu Asp Arg Tyr Tyr Arg Glu Asn Met His Arg Tyr Pro Asn Gln
145   150   155   160

Val Tyr Tyr Arg Pro Met Asp Glu Tyr Ser Asn Gln Asn Asn Phe Val
            165   170   175

His Asp Cys Val Asn Ile Thr Ile Lys Gln His Thr Val Thr Thr Thr
            180   185   190

Thr Lys Gly Glu Asn Phe Thr Glu Thr Asp Val Lys Met Met Glu Arg
        195   200   205

Val Val Glu Gln Met Cys Ile Thr Gln Tyr Glu Arg Glu Ser Gln Ala
    210   215   220

Tyr Tyr Gln Arg Gly Ser Ser Met Val Leu Phe Ser Ser Pro Pro Val
225   230   235   240

Ile Leu Leu Ile Ser Phe Leu Ile Phe Leu Ile Val Gly
            245   250

<210> 22
<211> 253
<212> PRT
<213> Gorilla

<400> 22

Met Ala Asn Leu Gly Cys Trp Met Leu Val Leu Phe Val Ala Thr Trp
1   5   10   15

Ser Asp Leu Gly Leu Cys Lys Lys Arg Pro Lys Pro Gly Gly Trp Asn
            20   25   30

Thr Gly Gly Ser Arg Tyr Pro Gly Gln Gly Ser Pro Gly Gly Asn Arg
        35   40   45

```
Tyr Pro Pro Gln Gly Gly Gly Gly Trp Gly Gln Pro His Gly Gly Gly
    50              55                  60

Trp Gly Gln Pro His Gly Gly Gly Trp Gly Gln Pro His Gly Gly Gly
65              70                  75                  80

Trp Gly Gln Pro His Gly Gly Gly Trp Gly Gln Gly Gly Gly Thr His
                85              90                  95

Ser Gln Trp Asn Lys Pro Ser Lys Pro Lys Thr Asn Met Lys His Met
            100             105             110

Ala Gly Ala Ala Ala Ala Gly Ala Val Val Gly Gly Leu Gly Gly Tyr
        115             120             125

Met Leu Gly Ser Ala Met Ser Arg Pro Ile Ile His Phe Gly Ser Asp
    130             135             140

Tyr Glu Asp Arg Tyr Tyr Arg Glu Asn Met His Arg Tyr Pro Asn Gln
145             150             155             160

Val Tyr Tyr Arg Pro Met Asp Gln Tyr Ser Asn Gln Asn Asn Phe Val
            165             170             175

His Asp Cys Val Asn Ile Thr Ile Lys Gln His Thr Val Thr Thr Thr
            180             185             190

Thr Lys Gly Glu Asn Phe Thr Glu Thr Asp Val Lys Met Met Glu Arg
        195             200             205

Val Val Glu Gln Met Cys Ile Thr Gln Tyr Glu Arg Glu Ser Gln Ala
    210             215             220

Tyr Tyr Gln Arg Gly Ser Ser Met Val Leu Phe Ser Ser Pro Pro Val
225             230             235             240

Ile Leu Leu Ile Ser Phe Leu Ile Phe Leu Ile Val Gly
            245             250
```

```
<210>  23
<211>  254
<212>  PRT
<213>  Chimpanzee

<400>  23
```

Met Ala Asn Leu Gly Cys Trp Met Leu Val Leu Phe Val Ala Thr Trp
1   5   10   15

Ser Asp Leu Gly Leu Cys Lys Lys Arg Pro Lys Pro Gly Gly Trp Asn
            20   25   30

Thr Gly Gly Ser Arg Tyr Pro Gly Gln Gly Ser Pro Gly Gly Asn Arg
            35   40   45

Tyr Pro Pro Gln Gly Gly Gly Gly Trp Gly Gln Pro His Gly Gly Gly
    50   55   60

Trp Gly Gln Pro His Gly Gly Gly Trp Gly Gln Pro His Gly Gly Gly
65   70   75   80

Trp Gly Gln Pro His Gly Gly Gly Trp Gly Gln Gly Gly Gly Thr His
              85   90   95

Ser Gln Trp Asn Lys Pro Ser Lys Pro Lys Thr Asn Met Lys His Met
            100   105   110

Ala Gly Ala Ala Ala Ala Gly Ala Val Val Gly Gly Leu Gly Gly Tyr
    115   120   .125

Met Leu Gly Ser Ala Met Ser Arg Pro Ile Ile His Phe Gly Ser Asp
    130   135   140

Tyr Glu Asp Arg Tyr Tyr Arg Glu Asn Met His Arg Tyr Pro Asn Gln
145   150   155   160

Val Tyr Tyr Arg Pro Met Asp Gln Tyr Ser Ser Gln Asn Asn Phe Val
            165   170   175

His Asp Cys Val Asn Ile Thr Ile Lys Gln His Thr Val Thr Thr Thr
            180   185   190

Thr Lys Gly Glu Asn Phe Thr Glu Thr Asp Val Lys Met Met Glu Arg
            195   200   205

Val Val Glu Gln Met Cys Ile Thr Gln Tyr Glu Arg Glu Ser Gln Ala
    210   215   220

Tyr Tyr Gln Arg Gly Ser Ser Met Val Leu Phe Ser Ser Pro Pro Val
225   230   235   240

Ile Leu Leu Ile Ser Phe Leu Ile Phe Leu Leu Ile Val Gly

245   250

<210> 24
<211> 254
<212> PRT
<213> Mus sp.

<400> 24

Met Ala Asn Leu Gly Tyr Trp Leu Leu Ala Leu Phe Val Thr Met Trp
1   5   10   15

Thr Asp Val Gly Leu Cys Lys Lys Arg Pro Lys Pro Gly Gly Trp Asn
            20   25   30

Thr Gly Gly Ser Arg Tyr Pro Gly Gln Gly Ser Pro Gly Gly Asn Arg
        35   40   45

Tyr Pro Pro Gln Gly Gly Thr Trp Gly Gln Pro His Gly Gly Gly Trp
    50   55   60

Gly Gln Pro His Gly Gly Ser Trp Gly Gln Pro Pro Gly Gly Ser Trp
65   70   75   80

Gly Gln Pro His Gly Gly Gly Trp Gly Gln Gly Gly Gly Thr His Asn
                85   90   95

Gln Trp Asn Lys Pro Ser Lys Pro Lys Thr Asn Leu Lys His Val Ala
            100   105   110

Gly Ala Ala Ala Ala Gly Ala Val Val Gly Gly Leu Gly Gly Tyr Met
        115   120   125

Leu Gly Ser Ala Met Ser Arg Pro Met Ile His Phe Gly Asn Asp Trp
    130   135   140

Glu Asp Arg Tyr Tyr Arg Glu Asn Met Tyr Arg Tyr Pro Asn Gln Val
145   150   155   160

Tyr Tyr Arg Pro Val Asp Gln Tyr Ser Asn Gln Asn Asn Phe Val His
                165   170   175

Asp Cys Val Asn Ile Thr Ile Lys Gln His Thr Val Thr Thr Thr Thr
            180   185   190

Lys Gly Glu Asn Phe Thr Glu Thr Asp Val Lys Met Met Glu Arg Val
        195   200   205

```
Val Glu Gln Met Cys Val Thr Gln Tyr Gln Lys Glu Ser Asp Ala Tyr
    210             215             220

Tyr Asp Gly Arg Arg Ser Ser Ser Thr Val Leu Phe Ser Ser Pro Pro
225             230             235             240

Val Ile Leu Leu Ile Ser Phe Leu Ile Phe Leu Ile Val Gly
            245             250
```

<210> 25
<211> 225
<212> PRT
<213> Rat

<400> 25

```
Gly Gly Trp Asn Thr Gly Gly Ser Arg Tyr Pro Gly Gln Gly Ser Pro
1               5               10              15

Gly Gly Asn Arg Tyr Pro Pro Gln Ser Gly Gly Thr Trp Gly Gln Pro
            20              25              30

His Gly Gly Gly Trp Gly Gln Pro His Gly Gly Gly Trp Gly Gln Pro
        35              40              45

His Gly Gly Gly Trp Gly Gln Pro His Gly Gly Gly Trp Ser Gln Gly
    50              55              60

Gly Gly Thr His Asn Gln Trp Asn Lys Pro Ser Lys Pro Lys Thr Asn
65              70              75              80

Leu Lys His Val Ala Gly Ala Ala Ala Gly Ala Val Val Gly Gly
            85              90              95

Leu Gly Gly Tyr Met Leu Gly Ser Ala Met Ser Arg Pro Met Leu His
            100             105             110

Phe Gly Asn Asp Trp Glu Asp Arg Tyr Tyr Arg Glu Asn Met Tyr Arg
        115             120             125

Tyr Pro Asn Gln Val Tyr Tyr Arg Pro Val Asp Gln Tyr Ser Asn Gln
    130             135             140

Asn Asn Phe Val His Asp Cys Val Asn Ile Thr Ile Lys Gln His Thr
145             150             155             160

Val Thr Thr Thr Thr Lys Gly Glu Asn Phe Thr Glu Thr Asp Val Lys
```

```
                        165                  170                  175

     Met Met Glu Arg Val Val Glu Gln Met Cys Val Thr Gln Tyr Gln Lys
                 180                  185                  190

     Glu Ser Gln Ala Tyr Tyr Asp Gly Arg Arg Ser Ser Ala Val Leu Phe
                 195                  200                  205

     Ser Ser Pro Pro Val Ile Leu Leu Ile Ser Leu Ile Phe Leu Ile Val
                 210                  215                  220

     Gly
     225


     <210>  26
     <211>  254
     <212>  PRT
     <213>  Syrian Hamster

     <400>  26

     Met Ala Asn Leu Ser Tyr Trp Leu Leu Ala Leu Phe Val Ala Met Trp
     1               5                   10                  15

     Thr Asp Val Gly Leu Cys Lys Lys Arg Pro Lys Pro Gly Gly Trp Asn
                 20                  25                  30

     Thr Gly Gly Ser Arg Tyr Pro Gly Gln Gly Ser Pro Gly Gly Asn Arg
                 35                  40                  45

     Tyr Pro Pro Gln Gly Gly Gly Thr Trp Gly Gln Pro His Gly Gly Gly
                 50                  55                  60

     Trp Gly Gln Pro His Gly Gly Gly Trp Gly Gln Pro His Gly Gly Gly
     65                  70                  75                  80

     Trp Gly Gln Pro His Gly Gly Gly Trp Gly Gln Gly Gly Gly Thr His
                 85                  90                  95

     Asn Gln Trp Asn Lys Pro Ser Lys Pro Lys Thr Asn Met Lys His Met
                 100                 105                 110

     Ala Gly Ala Ala Ala Ala Gly Ala Val Val Gly Gly Leu Gly Gly Tyr
                 115                 120                 125

     Met Leu Gly Ser Ala Met Ser Arg Pro Met Met His Phe Gly Asn Asp
                 130                 135                 140
```

```
Trp Glu Asp Arg Tyr Tyr Arg Glu Asn Met Asn Arg Tyr Pro Asn Gln
145                 150                 155                 160

Val Tyr Tyr Arg Pro Val Asp Gln Tyr Asn Asn Gln Asn Asn Phe Val
                165                 170                 175

His Asp Cys Val Asn Ile Thr Ile Lys Gln His Thr Val Thr Thr Tyr
            180                 185                 190

Thr Lys Gly Glu Asn Phe Thr Glu Thr Asp Ile Lys Ile Met Glu Arg
        195                 200                 205

Val Val Glu Gln Met Cys Thr Thr Gln Tyr Gln Lys Glu Ser Gln Ala
    210                 215                 220

Tyr Tyr Asp Gly Arg Arg Ser Ser Ala Val Leu Phe Ser Ser Pro Pro
225                 230                 235                 240

Val Ile Leu Leu Ile Ser Phe Leu Ile Phe Leu Met Val Gly
                245                 250
```

```
<210>   27
<211>   258
<212>   PRT
<213>   Mink

<400>   27
```

```
Met Val Lys Ser His Ile Gly Ser Trp Leu Leu Val Leu Phe Val Ala
1               5               10                  15

Thr Trp Ser Asp Ile Gly Phe Cys Lys Lys Arg Pro Lys Pro Gly Gly
            20                  25                  30

Gly Trp Asn Thr Gly Gly Ser Arg Tyr Pro Gly Gln Gly Ser Pro Gly
        35                  40                  45

Gly Asn Arg Tyr Pro Pro Gln Gly Gly Gly Gly Trp Gly Gln Pro His
    50                  55                  60

Gly Gly Gly Trp Gly Gln Pro His Gly Gly Gly Trp Gly Gln Pro His
65                  70                  75                  80

Gly Gly Gly Trp Gly Gln Pro His Gly Gly Gly Gly Trp Gly Gln Gly
                85                  90                  95

Gly Gly Ser His Gly Gln Trp Gly Lys Pro Ser Lys Pro Lys Thr Asn
```

```
                    100                 105                 110

        Met Lys His Val Ala Gly Ala Ala Ala Ala Gly Ala Val Val Gly Gly
            115                 120                 125

        Leu Gly Gly Tyr Met Leu Gly Ser Ala Met Ser Arg Pro Leu Ile His
            130                 135                 140

        Phe Gly Asn Asp Tyr Glu Asp Arg Tyr Tyr Arg Glu Asn Met Tyr Arg
        145                 150                 155                 160

        Tyr Pro Asn Gln Val Tyr Tyr Lys Pro Val Asp Gln Tyr Ser Asn Gln
                        165                 170                 175

        Asn Asn Phe Val His Asp Cys Val Asn Ile Thr Val Lys Gln His Thr
                    180                 185                 190

        Val Thr Thr Thr Thr Lys Gly Glu Asn Phe Thr Glu Thr Asp Met Lys
                195                 200                 205

        Ile Met Glu Arg Val Val Glu Gln Met Cys Val Thr Gln Tyr Gln Arg
            210                 215                 220

        Glu Ser Glu Ala Ala Tyr Tyr Gln Arg Gly Ala Ser Ala Ile Leu Phe
        225                 230                 235                 240

        Ser Pro Pro Pro Val Ile Leu Leu Ile Ser Leu Leu Ile Leu Leu Ile
                        245                 250                 255

        Val Gly


        <210>   28
        <211>   256
        <212>   PRT
        <213>   Sheep

        <400>   28

        Met Val Lys Ser His Ile Gly Ser Trp Ile Leu Val Leu Phe Val Ala
        1               5                   10                  15

        Met Trp Ser Asp Val Gly Leu Cys Lys Lys Arg Pro Lys Pro Gly Gly
                        20                  25                  30

        Gly Trp Asn Thr Gly Gly Ser Arg Tyr Pro Gly Gln Gly Ser Pro Gly
                35                  40                  45
```

```
Gly Asn Arg Tyr Pro Pro Gln Gly Gly Gly Gly Trp Gly Gln Pro His
    50              55              60

Gly Gly Gly Trp Gly Gln Pro His Gly Gly Gly Trp Gly Gln Pro His
65              70              75              80

Gly Gly Gly Trp Gly Gln Pro His Gly Gly Gly Gly Trp Gly Gln Gly
                85              90              95

Gly Ser His Ser Gln Trp Asn Lys Pro Ser Lys Pro Lys Thr Asn Met
            100             105             110

Lys His Val Ala Gly Ala Ala Ala Ala Gly Ala Val Val Gly Gly Leu
        115             120             125

Gly Gly Tyr Met Leu Gly Ser Ala Met Ser Arg Pro Leu Ile His Phe
    130             135             140

Gly Asn Asp Tyr Glu Asp Arg Tyr Tyr Arg Glu Asn Met Tyr Arg Tyr
145             150             155             160

Pro Asn Gln Val Tyr Tyr Arg Pro Val Asp Arg Tyr Ser Asn Gln Asn
                165             170             175

Asn Phe Val His Asp Cys Val Asn Ile Thr Val Lys Gln His Thr Val
            180             185             190

Thr Thr Thr Thr Lys Gly Glu Asn Phe Thr Glu Thr Asp Ile Lys Ile
            195             200             205

Met Glu Arg Val Val Glu Gln Met Cys Ile Thr Gln Tyr Gln Arg Glu
    210             215             220

Ser Gln Ala Tyr Tyr Gln Arg Gly Ala Ser Val Ile Leu Phe Ser Ser
225             230             235             240

Pro Pro Val Ile Leu Leu Ile Ser Phe Leu Ile Phe Leu Ile Val Gly
                245             250             255

<210>  29
<211>  256
<212>  PRT
<213>  Goat

<400>  29

Met Val Lys Ser His Ile Gly Ser Trp Ile Leu Val Leu Phe Val Ala
```

```
1                    5                    10                   15

Met Trp Ser Asp Val Gly Leu Cys Lys Lys Arg Pro Lys Pro Gly Gly
            20                  25                  30

Gly Trp Asn Thr Gly Gly Ser Arg Tyr Pro Gly Gln Gly Ser Pro Gly
            35                  40                  45

Gly Asn Arg Tyr Pro Pro Gln Gly Gly Gly Gly Trp Gly Gln Pro His
            50                  55                  60

Gly Gly Gly Trp Gly Gln Pro His Gly Gly Gly Trp Gly Gln Pro His
65                  70                  75                  80

Gly Gly Gly Trp Gly Gln Pro His Gly Gly Gly Gly Trp Gly Gln Gly
                    85                  90                  95

Gly Ser His Ser Asp Trp Asn Lys Pro Ser Lys Pro Lys Thr Asn Met
            100                 105                 110

Lys His Val Ala Gly Ala Ala Ala Gly Ala Val Val Gly Gly Leu
            115                 120                 125

Gly Gly Tyr Met Leu Gly Ser Ala Met Ser Arg Pro Leu Ile His Phe
            130                 135                 140

Gly His Asp Tyr Glu Asp Arg Tyr Tyr Arg Glu Asn Met Tyr Arg Tyr
145                 150                 155                 160

Pro Asn Gln Val Tyr Tyr Arg Pro Val Asp Gln Tyr Ser His Gln Asn
                    165                 170                 175

Asn Phe Val His Asp Cys Val Asn Ile Thr Val Lys Gln His Thr Val
            180                 185                 190

Thr Thr Thr Thr Lys Gly Glu Asn Phe Thr Glu Thr Asp Ile Lys Ile
            195                 200                 205

Met Glu Arg Val Val Glu Gln Met Cys Ile Thr Gln Tyr Gln Arg Glu
210                 215                 220

Ser Gln Ala Tyr Tyr Gln Arg Gly Ala Ser Val Ile Leu Phe Ser Pro
225                 230                 235                 240

Pro Pro Val Ile Leu Leu Ile Ser Leu Leu Ile Leu Leu Ile Val Gly
                    245                 250                 255
```

<210> 30
<211> 264
<212> PRT
<213> Cow

<400> 30

Met Val Lys Ser His Ile Gly Ser Trp Ile Leu Val Leu Phe Val Ala
1   5   10   15

Met Trp Ser Asp Val Gly Leu Cys Lys Lys Arg Pro Lys Pro Gly Gly
            20   25   30

Gly Trp Asn Thr Gly Gly Ser Arg Tyr Pro Gly Gln Gly Ser Pro Gly
        35   40   45

Gly Asn Arg Tyr Pro Pro Gln Gly Gly Gly Gly Trp Gly Gln Pro His
    50   55   60

Gly Gly Gly Trp Gly Gln Pro His Gly Gly Gly Trp Gly Gln Pro His
65   70   75   80

Gly Gly Gly Trp Gly Gln Pro His Gly Gly Gly Trp Gly Gln Pro His
                85   90   95

Gly Gly Gly Gly Trp Gly Gln Gly Gly Thr His Gly Gln Trp Asn Lys
            100   105   110

Pro Ser Lys Pro Lys Thr Asn Met Lys His Val Ala Gly Ala Ala Ala
        115   120   125

Ala Gly Ala Val Val Gly Gly Leu Gly Gly Tyr Met Leu Gly Ser Ala
        130   135   140

Met Ser Arg Pro Leu Ile His Phe Gly Ser Asp Tyr Glu Asp Arg Tyr
145   150   155   160

Tyr Arg Glu Asn Met His Arg Tyr Pro Asn Gln Val Tyr Tyr Arg Pro
                165   170   175

Val Asp Gln Tyr Ser Asn Gln Asn Asn Phe Val His Asp Cys Val Asn
            180   185   190

Ile Thr Val Lys Glu His Thr Val Thr Thr Thr Lys Gly Glu Asn
            195   200   . 205

```
Phe Thr Glu Thr Asp Ile Lys Met Met Glu Arg Val Val Glu Gln Met
    210             215             220
```

```
Cys Ile Thr Gln Tyr Gln Arg Glu Ser Gln Ala Tyr Tyr Gln Arg Gly
225             230             235             240
```

```
Ala Ser Val Ile Leu Phe Ser Ser Pro Pro Val Ile Leu Leu Ile Ser
            245             250             255
```

```
Phe Leu Ile Phe Leu Ile Val Gly
            260
```

<210> 31
<211> 263
<212> PRT
<213> Greater Kudu

<400> 31

```
Met Val Lys Ser His Ile Gly Ser Trp Ile Leu Val Leu Phe Val Ala
1               5               10              15
```

```
Met Trp Ser Asp Val Ala Leu Cys Lys Lys Arg Pro Lys Pro Gly Gly
            20              25              30
```

```
Gly Trp Asn Thr Gly Gly Ser Arg Tyr Pro Gly Gln Gly Ser Pro Gly
        35              40              45
```

```
Gly Asn Arg Tyr Pro Ser Gln Gly Gly Gly Gly Trp Gly Gln Pro His
    50              55              60
```

```
Gly Gly Gly Trp Gly Gln Pro His Gly Gly Gly Trp Gly Gln Pro His
65              70              75              80
```

```
Gly Gly Gly Trp Gly Gln Pro His Gly Gly Gly Trp Gly Gln Pro His
            85              90              95
```

```
Gly Gly Gly Gly Trp Gly Gln Gly Gly Thr His Gly Gln Trp Asn Lys
            100             105             110
```

```
Pro Ser Lys Lys Thr Asn Met Lys His Val Ala Gly Ala Ala Ala Ala
        115             120             125
```

```
Gly Ala Val Val Gly Gly Leu Gly Gly Tyr Met Leu Gly Ser Ala Met
    130             135             140
```

```
Ser Arg Pro Leu Ile His Phe Gly Ser Asp Tyr Glu Asp Arg Tyr Tyr
145             150             155             160
```

Arg Glu Asn Met Tyr Arg Tyr Pro Asn Gln Val Tyr Tyr Arg Pro Val
            165   170   175

Asp Gln Tyr Ser Asn Gln Asn Asn Phe Val His Asp Val Asn Asn Ile
            180   185   190

Thr Val Lys Gln His Thr Val Thr Thr Thr Lys Gly Glu Asn Phe
            195   200   205

Thr Glu Thr Asp Ile Lys Met Met Glu Arg Val Val Glu Gln Met Cys
     210   215   220

Ile Thr Gln Tyr Gln Arg Glu Ser Glu Ala Tyr Tyr Gln Arg Gly Ala
225   230   235   240

Ser Val Ile Leu Phe Ser Ser Pro Pro Val Ile Leu Leu Ile Ser Phe
            245   250   255

Leu Ile Phe Leu Ile Val Gly
            260

<210> 32
<211> 253
<212> PRT
<213> Artificial Sequence

<220>
<223> synthetic

<220>
<221> misc_feature
<222> (121)..(122)
<223> Amino acid residues 121, 122, 128, 129, and 130 are Val, Val, Tyr,
      Met, and Leu, respectively, in which one to five of residues 121,
      122, 128, 129, and 130, is substituted with Pro, Asp, Glu, Lys, Gly,
      or Ser.

<220>
<221> misc_feature
<222> (128)..(130)
<223> Amino acid residues 121, 122, 128, 129, and 130 are Val, Val, Tyr,
      Met, and Leu, respectively, in which one to five of residues 121,
      122, 128, 129, and 130 is substituted with Pro, Asp, Glu, Lys, Gly,
      or Ser.

<400> 32

Met Ala Asn Leu Gly Cys Trp Met Leu Val Leu Phe Val Ala Thr Trp
1   5   10   15

```
Ser Asp Leu Gly Leu Cys Lys Lys Arg Pro Lys Pro Gly Gly Trp Asn
         20                  25                  30


Thr Gly Gly Ser Arg Tyr Pro Gly Gln Gly Ser Pro Gly Gly Asn Arg
         35                  40                  45


Tyr Pro Pro Gln Gly Gly Gly Gly Trp Gly Gln Pro His Gly Gly Gly
    50                  55                  60


Trp Gly Gln Pro His Gly Gly Gly Trp Gly Gln Pro His Gly Gly Gly
65                  70                  75                  80


Trp Gly Gln Pro His Gly Gly Gly Trp Gly Gln Gly Gly Gly Thr His
                 85                  90                  95


Ser Gln Trp Asn Lys Pro Ser Lys Pro Lys Thr Asn Met Lys His Met
             100                 105                 110


Ala Gly Ala Ala Ala Ala Gly Ala Xaa Xaa Gly Gly Leu Gly Gly Xaa
         115                 120                 125


Xaa Xaa Gly Ser Ala Met Ser Arg Pro Ile Ile His Phe Gly Ser Asp
    130                 135                 140


Tyr Glu Asp Arg Tyr Tyr Arg Glu Asn Met His Arg Tyr Pro Asn Gln
145                 150                 155                 160


Val Tyr Tyr Arg Pro Met Asp Glu Tyr Ser Asn Gln Asn Asn Phe Val
             165                 170                 175


His Asp Cys Val Asn Ile Thr Ile Lys Gln His Thr Val Thr Thr Thr
         180                 185                 190


Thr Lys Gly Glu Asn Phe Thr Glu Thr Asp Val Lys Met Met Glu Arg
         195                 200                 205


Val Val Glu Gln Met Cys Ile Thr Gln Tyr Glu Arg Glu Ser Gln Ala
    210                 215                 220


Tyr Tyr Gln Arg Gly Ser Ser Met Val Leu Phe Ser Ser Pro Pro Val
225                 230                 235                 240


Ile Leu Leu Ile Ser Phe Leu Ile Phe Leu Ile Val Gly
             245                 250
```

```
<210>  33
<211>  264
<212>  PRT
<213>  Artificial Sequence

<220>
<223>  synthetic

<220>
<221>  misc_feature
<222>  (132)..(133)
<223>  Amino acid residues 132, 133, 139, 140, and 141 are Val, Val, Tyr,
       Met, and Leu, respectively, in which one to five of residues 132,
       133, 139, 140, and 141 is substituted with Pro, Asp, Glu, Lys,
       Gly, or Ser.

<220>
<221>  misc_feature
<222>  (139)..(141)
<223>  Amino acid residues 132, 133, 139, 140, and 141 are Val, Val, Tyr,
       Met, and Leu, respectively, in which one to five of residues 132,
       133, 139, 140, and 141 is substituted with Pro, Asp, Glu, Lys,
       Gly, or Ser.

<400>  33

Met Val Lys Ser His Ile Gly Ser Trp Ile Leu Val Leu Phe Val Ala
1               5                   10                  15


Met Trp Ser Asp Val Gly Leu Cys Lys Lys Arg Pro Lys Pro Gly Gly
            20                  25                  30


Gly Trp Asn Thr Gly Gly Ser Arg Tyr Pro Gly Gln Gly Ser Pro Gly
        35                  40                  45


Gly Asn Arg Tyr Pro Pro Gln Gly Gly Gly Gly Trp Gly Gln Pro His
    50                  55                  60


Gly Gly Gly Trp Gly Gln Pro His Gly Gly Gly Trp Gly Gln Pro His
65                  70                  75                  80


Gly Gly Gly Trp Gly Gln Pro His Gly Gly Gly Trp Gly Gln Pro His
                85                  90                  95


Gly Gly Gly Gly Trp Gly Gln Gly Gly Thr His Gly Gln Trp Asn Lys
            100                 105                 110


Pro Ser Lys Pro Lys Thr Asn Met Lys His Val Ala Gly Ala Ala Ala
        115                 120                 125


Ala Gly Ala Xaa Xaa Gly Gly Leu Gly Gly Xaa Xaa Xaa Gly Ser Ala
```

```
           130                    135                    140


Met Ser Arg Pro Leu Ile His Phe Gly Ser Asp Tyr Glu Asp Arg Tyr
145                 150                 155                 160


Tyr Arg Glu Asn Met His Arg Tyr Pro Asn Gln Val Tyr Tyr Arg Pro
                165                 170                 175


Val Asp Gln Tyr Ser Asn Gln Asn Asn Phe Val His Asp Cys Val Asn
                180                 185                 190


Ile Thr Val Lys Glu His Thr Val Thr Thr Thr Thr Lys Gly Glu Asn
            195                 200                 205


Phe Thr Glu Thr Asp Ile Lys Met Met Glu Arg Val Val Glu Gln Met
    210                 215                 220


Cys Ile Thr Gln Tyr Gln Arg Glu Ser Gln Ala Tyr Tyr Gln Arg Gly
225                 230                 235                 240


Ala Ser Val Ile Leu Phe Ser Ser Pro Pro Val Ile Leu Leu Ile Ser
            245                 250                 255


Phe Leu Ile Phe Leu Ile Val Gly
            260
```

<210> 34
<211> 65
<212> PRT
<213> human PrP

<220>
<221> misc_feature
<222> (1)..(10)
<223> Amino acid residues 7-10 either are present, together as all Lys
      or all Asp, or are all absent. When residues 7- 10 are present then
      any one or all of residues 1-6 can either be absent or present
      as Lys or Asp to form, in combination with residues 7-10, a N-
      terminal polylysine or polyaspartate segment of 4 to 10 residues in
      length.

<220>
<221> misc_feature
<222> (42)..(43)
<223> Amino acid residues 42, 43, 49, 50, and 51 are Val, Val, Tyr, Met,
      and Leu, respectively, in which zero or one to five of residues
      42, 43, 49, 50, and 51 is substituted with Pro, Asp, Glu, Lys,
      Gly, or Ser. The C-terminal Asp residue may be amidated.

<220>

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<221>   misc_feature
<222>   (49)..(51)
<223>   Amino acid residues 42, 43, 49, 50, and 51 are Val, Val, Tyr, Met,
        and Leu, respectively, in which zero or one to five of residues
        42, 43, 49, 50, and 51 is substituted with Pro, Asp, Glu, Lys,
        Gly, or Ser.  The C-terminal Asp residue may be amidated.


<400>   34

Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Gly Gln Gly Gly Gly Thr
1               5                   10                  15


His Ser Gln Trp Asn Lys Pro Ser Lys Pro Lys Thr Asn Met Lys His
            20                  25                  30


Met Ala Gly Ala Ala Ala Ala Gly Ala Xaa Xaa Gly Gly Leu Gly Gly
        35                  40                  45


Xaa Xaa Xaa Gly Ser Ala Met Ser Arg Pro Ile Ile His Phe Gly Ser
    50                  55                  60


Asp
65


<210>   35
<211>   120
<212>   PRT
<213>   Artificial Sequence

<220>
<223>   synthetic

<220>
<221>   misc_feature
<222>   (1)..(10)
<223>   Amino acid residues 7-10 either are present, together as all Lys
        or all Asp, or are all absent.  When residues 7-10 are present then
        any one or all of residues 1-6 can either be absent or present
        as Lys or Asp to form, in combination with residues 7-10, a N-
        terminal polylysine or polyaspartate segment of 4 to 10 residues in
        length.


<220>
<221>   misc_feature
<222>   (42)..(43)
<223>   Amino acid residues 42, 43, 49, 50, 51 and residues 97, 98, 104,
        105, 106 are the same and are Val, Val, Tyr, Met, and Leu,
        respectively, in which zero or one to five of residues 42, 43, 49, 50,
        and 51 and the same zero or one to five of residues 97, 98, 104, 105,
        and 106 is substituted with Pro, Asp, Glu, Lys, Gly, or Ser.


<220>
<221>   misc_feature
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-31-

```
<222>  (49)..(51)
<223>  Amino acid residues 42, 43, 49, 50, 51 and residues 97, 98, 104,
       105, 106 are the same and are Val, Val, Tyr, Met, and Leu,
       respectively, in which zero or one to five of residues 42, 43, 49, 50,
       and 51 and the same zero or one to five of residues 97, 98, 104, 105,
       and 106 is substituted with Pro, Asp, Glu, Lys, Gly, or Ser.


<220>
<221>  misc_feature
<222>  (97)..(98)
<223>  Amino acid residues 42, 43, 49, 50, 51 and residues 97, 98, 104,
       105, 106 are the same and are Val, Val, Tyr, Met, and Leu,
       respectively, in which zero or one to five of residues 42, 43, 49, 50,
       and 51 and the same zero or one to five of residues 97, 98, 104, 105,
       and 106 is substituted with Pro, Asp, Glu, Lys, Gly, or Ser.


<220>
<221>  misc_feature
<222>  (104)..(106)
<223>  Amino acid residues 42, 43, 49, 50, 51 and residues 97, 98, 104,
       105, 106 are the same and are Val, Val, Tyr, Met, and Leu,
       respectively, in which zero or one to five of residues 42, 43, 49, 50,
       and 51 and the same zero or one to five of residues 97, 98, 104, 105,
       and 106 is substituted with Pro, Asp, Glu, Lys, Gly, or Ser.


<220>
<221>  misc_feature
<223>  The C-terminal Asp residue may be amidated.


<400>  35

Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Gly Gln Gly Gly Gly Thr
1               5                   10                  15


His Ser Gln Trp Asn Lys Pro Ser Lys Pro Lys Thr Asn Met Lys His
            20                  25                  30


Met Ala Gly Ala Ala Ala Ala Gly Ala Xaa Xaa Gly Gly Leu Gly Gly
        35                  40                  45


Xaa Xaa Xaa Gly Ser Ala Met Ser Arg Pro Ile Ile His Phe Gly Ser
    50                  55                  60


Asp Gly Gln Gly Gly Gly Thr His Ser Gln Trp Asn Lys Pro Ser Lys
65                  70                  75                  80


Pro Lys Thr Asn Met Lys His Met Ala Gly Ala Ala Ala Ala Gly Ala
                85                  90                  95


Xaa Xaa Gly Gly Leu Gly Gly Xaa Xaa Xaa Gly Ser Ala Met Ser Arg
            100                 105                 110
```

```
Pro Ile Ile His Phe Gly Ser Asp
         115                 120


<210>  36
<211>  65
<212>  PRT
<213>  Artificial Sequence

<220>
<223>  synthetic

<220>
<221>  misc_feature
<222>  (32)..(33)
<223>  Amino acid residues 32, 33, 39, 40, and 41 are Val, Val, Tyr, Met,
       and Leu, respectively, in which zero or one to five of residues
       32, 33, 39, 40, and 41 is substituted with Pro, Asp, Glu, Lys,
       Gly, or Ser.


<220>
<221>  misc_feature
<222>  (39)..(41)
<223>  Amino acid residues 32, 33, 39, 40, and 41 are Val, Val, Tyr, Met,
       and Leu, respectively, in which zero or one to five of residues
       32, 33, 39, 40, and 41 is substituted with Pro, Asp, Glu, Lys, Gly,
       or Ser.


<220>
<221>  misc_feature
<222>  (56)..(65)
<223>  Amino acid residues 56-59 are all Lys or all Asp or are all absent.
       When all residues 56-59 are present, then any one or all of the
       residues 60-65 can either be absent or present as Lys or Asp to
       form, in combination with residues 56-59, a C-terminal polylysine
       or polyaspartate segment of 4 to 10 residues in length.


<400>  36

Gly Gln Gly Gly Gly Thr His Ser Gln Trp Asn Lys Pro Ser Lys Pro
1               5                   10                  15


Lys Thr Asn Met Lys His Met Ala Gly Ala Ala Ala Ala Gly Ala Xaa
            20                  25                  30


Xaa Gly Gly Leu Gly Gly Xaa Xaa Xaa Gly Ser Ala Met Ser Arg Pro
        35                  40                  45


Ile Ile His Phe Gly Ser Asp Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa
    50                  55                  60


Xaa
```

65

```
<210>  37
<211>  120
<212>  PRT
<213>  Artificial Sequence

<220>
<223>  synthetic

<220>
<221>  misc_feature
<222>  (32)..(33)
<223>  Amino acid residues 32, 33, 39, 40, 41 and residues 87, 88, 94, 95,
       96 are the same and are Val, Val, Tyr, Met, and Leu, respectively,
       in which zero or one to five of residues 32, 33, 39, 40, and
       41 and the same zero, one to five of residues 87, 88, 94, 95, and
       96 is substituted with Pro, Asp, Glu, Lys, Gly, or Ser.

<220>
<221>  misc_feature
<222>  (39)..(41)
<223>  Amino acid residues 32, 33, 39, 40, 41 and residues 87, 88, 94, 95,
       96 are the same and are Val, Val, Tyr, Met, and Leu, respectively
       in which zero or one to five of residues 32, 33, 39, 40, and
       41 and the same zero, one to five of residues 87, 88, 94, 95, and
       96 is substituted with Pro, Asp, Glu, Lys, Gly, or Ser.

<220>
<221>  misc_feature
<222>  (87)..(88)
<223>  Amino acid residues 32, 33, 39, 40, 41 and residues 87, 88, 94, 95,
       96 are the same and are Val, Val, Tyr, Met, and Leu, respectively,
       in which zero or one to five of residues 32, 33, 39, 40, and
       41 and the same zero, one to five of residues 87, 88, 94, 95, and
       96 is substituted with Pro, Asp, Glu, Lys, Gly, or Ser.

<220>
<221>  misc_feature
<222>  (94)..(96)
<223>  Amino acid residues 32, 33, 39, 40, 41 and residues 87, 88, 94, 95,
       96 are the same and are Val, Val, Tyr, Met, and Leu, respectively,
       in which zero or one to five of residues 32, 33, 39, 40, and
       41 and the same zero, one to five of residues 87, 88, 94, 95, and
       96 is substituted with Pro, Asp, Glu, Lys, Gly, or Ser.

<220>
<221>  misc_feature
<222>  (111)..(120)
<223>  Amino acid residues 111-114 are all Lys or all Asp, or are all absent
       When all residues 111-114 are present, then any one or all
       of residues 115-120 can either be Lys or Asp to form, in combination
       with residues 111-114, a C-terminal polylysine or polyaspartate
       segment of 4-10 residues in length.
```

<400> 37

Gly Gln Gly Gly Gly Thr His Ser Gln Trp Asn Lys Pro Ser Lys Pro
1   5   10   15

Lys Thr Asn Met Lys His Met Ala Gly Ala Ala Ala Ala Gly Ala Xaa
                20   25   30

Xaa Gly Gly Leu Gly Gly Xaa Xaa Xaa Gly Ser Ala Met Ser Arg Pro
            35   40   45

Ile Ile His Phe Gly Ser Asp Gly Gln Gly Gly Gly Thr His Ser Gln
        50   55   60

Trp Asn Lys Pro Ser Lys Pro Lys Thr Asn Met Lys His Met Ala Gly
65   70   75   80

Ala Ala Ala Ala Gly Ala Xaa Xaa Gly Gly Leu Gly Gly Xaa Xaa Xaa
                85   90   95

Gly Ser Ala Met Ser Arg Pro Ile Ile His Phe Gly Ser Asp Xaa Xaa
            100   105   110

Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa
        115   120

<210> 38
<211> 75
<212> PRT
<213> Artificial Sequence

<220>
<223> synthetic

<220>
<221> misc_feature
<222> (1)..(10)
<223> Amino acid residues 7-10 are all Lys or all Asp. Any one or all
       of residues 1-6 can either be absent or present as Lys or Asp to
       form, in combination with residues 7-10, a N-terminal polylysine
       or polyaspartate segment of 4 to 10 residues in length.

<220>
<221> misc_feature
<222> (42)..(43)
<223> Amino acid residues 42, 43, 49, 50, and 51 are Val, Val, Tyr, Met,
       and Leu, respectively, in which zero or one to five of residues
       42, 43, 49, 50, and 51 is substituted with Pro, Asp, Glu, Lys, Gly
       or Ser.

<220>

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<221>  misc_feature
<222>  (49)..(51)
<223>  Amino acid residues 42, 43, 49, 50, and 51 are Val, Val, Tyr, Met,
       and Leu, respectively, in which zero or one to five of residues
       42, 43, 49, 50, and 51 is substituted with Pro, Asp, Glu, Lys, Gly,
       or Ser.


<220>
<221>  misc_feature
<222>  (66)..(75)
<223>  Amino acids 66-69 are all Lys or all Asp.  Any one or all of residues
       70-75 can either be absent or present as Lys or Asp to form,
       in combination with residues 66-69, a C-terminal polylysine or
       polyaspartate segment of 4 to 10 residues in length.


<400>  38

Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Gly Gln Gly Gly Gly Thr
1               5                   10                  15


His Ser Gln Trp Asn Lys Pro Ser Lys Pro Lys Thr Asn Met Lys His
            20                  25                  30


Met Ala Gly Ala Ala Ala Ala Gly Ala Xaa Xaa Gly Gly Leu Gly Gly
        35                  40                  45


Xaa Xaa Xaa Gly Ser Ala Met Ser Arg Pro Ile Ile His Phe Gly Ser
    50                  55                  60


Asp Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa
65                  70                  75


<210>  39
<211>  130
<212>  PRT
<213>  Artificial Sequence

<220>
<223>  synthetic

<220>
<221>  misc_feature
<222>  (1)..(10)
<223>  Amino acid residues 7-10 are all Lys or all Asp.  Any one or all
       of residues 1-6 can either be absent or present as Lys or Asp to
       form, in combination with residues 7-10, a N-terminal polylysine
       or polyaspartate segment of 4 to 10 residues in length.


<220>
<221>  misc_feature
<222>  (42)..(43)
<223>  Amino acid residues 42, 43, 49, 50, 51 and residues 97, 98, 104,
       105, 106 are the same and are Val, Val, Tyr, Met, and Leu,
```

respectively, in which zero or one to five of residues 42, 43, 49, 50, and 51 and the same zero or one to five of residues 97, 98, 104, 105, and 106 is substituted with Pro, Asp, Glu, Lys, Gly, or Ser.

<220>
<221> misc_feature
<222> (49)..(51)
<223> Amino acid residues 42, 43, 49, 50, 51 and residues 97, 98, 104, 105, 106 are the same and are Val, Val, Tyr, Met, and Leu, respectively, in which zero or one to five of residues 42, 43, 49, 50, and 51 and the same zero or one to five of residues 97, 98, 104, 105, and 106 is substituted with Pro, Asp, Glu, Lys, Gly, or Ser.

<220>
<221> misc_feature
<222> (97)..(98)
<223> Amino acid residues 42, 43, 49, 50, 51 and residues 97, 98, 104, 105, 106 are the same and are Val, Val, Tyr, Met, and Leu, respectively, in which zero or one to five of residues 42, 43, 49, 50, and 51 and the same zero or one to five of residues 97, 98, 104, 105, and 106 is substituted with Pro, Asp, Glu, Lys, Gly, or Ser.

<220>
<221> misc_feature
<222> (104)..(106)
<223> Amino acid residues 42, 43, 49, 50, 51 and residues 97, 98, 104, 105, 106 are the same and are Val, Val, Tyr, Met, and Leu, respectively, in which zero or one to five of residues 42, 43, 49, 50, and 51 and the same zero or one to five of residues 97, 98, 104, 105, and 106 is substituted with Pro, Asp, Glu, Lys, Gly, or Ser.

<220>
<221> misc_feature
<222> (121)..(130)
<223> Amino acids 121-124 are all Lys or all Asp. Any one or all of residues 125-130 can either be absent or present as Lys or Asp to form, in combination with residues 121-124, a C-terminal polylysine or polyaspartate segment of 4 to 10 residues in length.

<400> 39

Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Gly Gln Gly Gly Gly Thr
1   5   10   15

His Ser Gln Trp Asn Lys Pro Ser Lys Pro Lys Thr Asn Met Lys His
            20   25   30

Met Ala Gly Ala Ala Ala Ala Gly Ala Xaa Xaa Gly Gly Leu Gly Gly
        35   40   45

Xaa Xaa Xaa Gly Ser Ala Met Ser Arg Pro Ile Ile His Phe Gly Ser
    50   55   60

```
Asp Gly Gln Gly Gly Gly Thr His Ser Gln Trp Asn Lys Pro Ser Lys
65                  70                  75                  80


Pro Lys Thr Asn Met Lys His Met Ala Gly Ala Ala Ala Ala Gly Ala
                85                  90                  95


Xaa Xaa Gly Gly Leu Gly Gly Xaa Xaa Xaa Gly Ser Ala Met Ser Arg
            100                 105             110


Pro Ile Ile His Phe Gly Ser Asp Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa
            115             120             125


Xaa Xaa
    130
```

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<210>   40
<211>   73
<212>   PRT
<213>   Artificial Sequence

<220>
<223>   synthetic

<220>
<221>   misc_feature
<222>   (1)..(10)
<223>   Amino acid residues 7-10 either are present, together as all Lys
        or all Asp, or are all absent.  When residues 7-10 are present then
        any one or all of residues 1-6 can either be absent or present
        as Lys or Asp to form, in combination with residues 7-10, a N-
        terrminal polylysine or polyaspartate segment of 4 to10 residues in
        length.


<220>
<221>   misc_feature
<222>   (50)..(51)
<223>   Amino acid residues 50, 51, 57, 58, and 59 are Val, Val, Tyr, Met,
        and Leu, respectively, in which zero or one to five of residues
        50, 51, 57, 58, and 59 is substituted with Pro, Asp, Glu, Lys, Gly,
        or Ser.  The C-terminal Asp residue may be amidated.


<220>
<221>   misc_feature
<222>   (57)..(59)
<223>   Amino acid residues 50, 51, 57, 58, and 59 are Val, Val, Tyr, Met,
        and Leu, respectively, in which zero or one to five of residues
        50, 51, 57, 58, and 59 is substituted with Pro, Asp, Glu, Lys, Gly,
        or Ser.  The C-terminal Asp residue may be amidated.


<400>   40

Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Gly Gln Pro His Gly Gly
```

```
1              5                   10                  15
```

```
Gly Gly Trp Gly Gln Gly Gly Thr His Gly Gln Trp Asn Lys Pro Ser
            20                  25              30
```

```
Lys Pro Lys Thr Asn Met Lys His Val Ala Gly Ala Ala Ala Ala Gly
            35              40              45
```

```
Ala Xaa Xaa Gly Gly Leu Gly Gly Xaa Xaa Xaa Gly Ser Ala Met Ser
    50                  55              60
```

```
Arg Pro Leu Ile His Phe Gly Asn Asp
65                  70
```

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<210>  41
<211>  136
<212>  PRT
<213>  Artificial Sequence

<220>
<223>  synthetic

<220>
<221>  misc_feature
<222>  (1)..(10)
<223>  Amino acid residues 7-10 either are present, together as all Lys
       or all Asp, or are all absent.  When residues 7-10 are present then
       any one or all of residues 1-6 can either be absent or present
       as Lys or Asp to form, in combination with residues 7-10, a N-
       terrminal polylysine or polyaspartate segment of 4 to10 residues in
       length.


<220>
<221>  misc_feature
<222>  (50)..(51)
<223>  Amino acid residues 50, 51, 57, 58, 59 and residues 113, 114, 120,
       121, 122 are the same and are Val, Val, Tyr, Met, and Leu,
       respectively, in which zero or one to five of residues 50, 51, 57, 58,
       59 and the same zero or one to five of residues 113, 114, 120, 121,
       122 is substituted with Pro, Asp, Glu, Lys, Gly, or Ser.


<220>
<221>  misc_feature
<222>  (57)..(59)
<223>  Amino acid residues 50, 51, 57, 58, 59 and residues 113, 114, 120,
       121, 122 are the same and are Val, Val, Tyr, Met, and Leu,
       respectively, in which zero or one to five of residues 50, 51, 57, 58,
       59 and the same zero or one to five of residues 113, 114, 120, 121,
       122 is substituted with Pro, Asp, Glu, Lys, Gly, or Ser.


<220>
<221>  misc_feature
<222>  (113)..(114)
```

<223> Amino acid residues 50, 51, 57, 58, 59 and residues 113, 114, 120,
       121, 122 are the same and are Val, Val, Tyr, Met, and Leu,
       respectively, in which zero or one to five of residues 50, 51, 57, 58,
       59 and the same zero or one to five of residues 113, 114, 120, 121,
       122 is substituted with Pro, Asp, Glu, Lys, Gly, or Ser.

<220>
<221> misc_feature
<222> (120)..(122)
<223> Amino acid residues 50, 51, 57, 58, 59 and residues 113, 114, 120,
       121, 122 are the same and are Val, Val, Tyr, Met, and Leu,
       respectively, in which zero or one to five of residues 50, 51, 57, 58,
       59 and the same zero or one to five of residues 113, 114, 120, 121,
       122 is substituted with Pro, Asp, Glu, Lys, Gly, or Ser.

<220>
<221> misc_feature
<223> The C-terminal Asp residue may be amidated.

<400> 41

Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Gly Gln Pro His Gly Gly
1   5   10   15

Gly Gly Trp Gly Gln Gly Gly Thr His Gly Gln Trp Asn Lys Pro Ser
            20   25   30

Lys Pro Lys Thr Asn Met Lys His Val Ala Gly Ala Ala Ala Ala Gly
        35   40   45

Ala Xaa Xaa Gly Gly Leu Gly Gly Xaa Xaa Xaa Gly Ser Ala Met Ser
    50   55   60

Arg Pro Leu Ile His Phe Gly Asn Asp Gly Gln Pro His Gly Gly Gly
65   70   75   80

Gly Trp Gly Gln Gly Gly Thr His Gly Gln Trp Asn Lys Pro Ser Lys
            85   90   95

Pro Lys Thr Asn Met Lys His Val Ala Gly Ala Ala Ala Ala Gly Ala
        100   105   110

Xaa Xaa Gly Gly Leu Gly Gly Xaa Xaa Xaa Gly Ser Ala Met Ser Arg
        115   120   125

Pro Leu Ile His Phe Gly Asn Asp
    130   135

<210> 42

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<211>   73
<212>   PRT
<213>   Artificial Sequence

<220>
<223>   synthetic

<220>
<221>   misc_feature
<222>   (40)..(41)
<223>   Amino acid residues 40, 41, 47, 48, and 49 are Val, Val, Tyr, Met,
        and Leu, respectively, in which zero or one to five of residues
        40, 41, 47, 48, and 49 is substituted with Pro, Asp, Glu, Lys,
        Gly, or Ser.

<220>
<221>   misc_feature
<222>   (47)..(49)
<223>   Amino acid residues 40, 41, 47, 48, and 49 are Val, Val, Tyr, Met,
        and Leu, respectively, in which zero or one to five of residues
        40, 41, 47, 48, and 49 is substituted with Pro, Asp, Glu, Lys,
        Gly, or Ser.

<220>
<221>   misc_feature
<222>   (64)..(73)
<223>   Amino acid residues 64-67 are all Lys or all Asp, or are all absent.
        When all residues 64-67 are present, then any one or all of
        residues 68-73 can either be absent or present as Lys or Asp to form,
        in combination with residues 64-67, a C-terminal polylysine
        or polyaspartate segment of 4 to 10 residues in length.


<400>   42

Gly Gln Pro His Gly Gly Gly Gly Trp Gly Gln Gly Gly Thr His Gly
1               5                   10                  15


Gln Trp Asn Lys Pro Ser Lys Pro Lys Thr Asn Met Lys His Val Ala
            20              25                  30


Gly Ala Ala Ala Ala Gly Ala Xaa Xaa Gly Gly Leu Gly Gly Xaa Xaa
        35                  40                  45


Xaa Gly Ser Ala Met Ser Arg Pro Leu Ile His Phe Gly Asn Asp Xaa
    50                  55                  60


Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa
65                  70


<210>   43
<211>   136
<212>   PRT
<213>   Artificial Sequence
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```
<220>
<223>  synthetic

<220>
<221>  misc_feature
<222>  (40)..(41)
<223>  Amino acid residues 40, 41, 47, 48, 49 and residues 103, 104, 110,
       111, 112 are the same and are Val, Val, Tyr, Met, and Leu,
       respectively in which zero or one to five of residues 40, 41, 47, 48,
       and 49 and the same zero or one to five of residues 103, 104, 110,
       111, and 112 is substituted with Pro, Asp, Glu, Lys, Gly, or Ser.

<220>
<221>  misc_feature
<222>  (47)..(49)
<223>  Amino acid residues 40, 41, 47, 48, 49 and residues 103, 104, 110,
       111, 112 are the same and are Val, Val, Tyr, Met, and Leu,
       respectively in which zero or one to five of residues 40, 41, 47, 48,
       and 49 and the same zero or one to five of residues 103, 104, 110,
       111, and 112 is substituted with Pro, Asp, Glu, Lys, Gly, or Ser.

<220>
<221>  misc_feature
<222>  (103)..(104)
<223>  Amino acid residues 40, 41, 47, 48, 49 and residues 103, 104, 110,
       111, 112 are the same and are Val, Val, Tyr, Met, and Leu,
       respectively in which zero or one to five of residues 40, 41, 47, 48,
       and 49 and the same zero or one to five of residues 103, 104, 110,
       111, and 112 is substituted with Pro, Asp, Glu, Lys, Gly, or Ser.

<220>
<221>  misc_feature
<222>  (110)..(112)
<223>  Amino acid residues 40, 41, 47, 48, 49 and residues 103, 104, 110,
       111, 112 are the same and are Val, Val, Tyr, Met, and Leu,
       respectively in which zero or one to five of residues 40, 41, 47, 48,
       and 49 and the same zero or one to five of residues 103, 104, 110,
       111, and 112 is substituted with Pro, Asp, Glu, Lys, Gly, or Ser.

<220>
<221>  misc_feature
<222>  (127)..(136)
<223>  Amino acid residues 127-130 are all Lys or all Asp, or are all absent.
       When all residues 127-130 are present, then any one or all
       of residues 131-136 can either be Lys or Asp to form, in combination
       with residues 127-130, a C-terminal polylysine or polyaspartate
       segment of 4 to 10 residues in length.

<400>  43

Gly Gln Pro His Gly Gly Gly Gly Trp Gly Gln Gly Gly Thr His Gly
1               5                   10                  15


Gln Trp Asn Lys Pro Ser Lys Pro Lys Thr Asn Met Lys His Val Ala
```

```
              20                    25                    30

Gly Ala Ala Ala Ala Gly Ala Xaa Xaa Gly Gly Leu Gly Gly Xaa Xaa
         35                    40                    45


Xaa Gly Ser Ala Met Ser Arg Pro Leu Ile His Phe Gly Asn Asp Gly
    50                   55                    60


Gln Pro His Gly Gly Gly Gly Trp Gly Gln Gly Gly Thr His Gly Gln
65                   70                    75                    80


Trp Asn Lys Pro Ser Lys Pro Lys Thr Asn Met Lys His Val Ala Gly
              85                    90                    95


Ala Ala Ala Ala Gly Ala Xaa Xaa Gly Gly Leu Gly Gly Xaa Xaa Xaa
         100                   105                   110


Gly Ser Ala Met Ser Arg Pro Leu Ile His Phe Gly Asn Asp Xaa Xaa
         115                   120                   125


Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa
    130                   135
```

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<210>  44
<211>  83
<212>  PRT
<213>  Artificial Sequence

<220>
<223>  synthetic

<220>
<221>  misc_feature
<222>  (1)..(10)
<223>  Amino acid residues 7-10 are all Lys or all Asp.  Any one or all
       of residues 1-6 can either be absent or present as Lys or Asp to
       form, in combination with residues 7-10, a N-terminal polylysine
       or polyaspartate segment of 4 to 10 residues in length.


<220>
<221>  misc_feature
<222>  (50)..(51)
<223>  Amino acid residues 50, 51, 57, 58 and 59 are Val, Val, Tyr, Met,
       and Leu, respectively, in which zero or one to five of residues
       50, 51, 57, 58, and 59 is substituted with Pro, Asp, Glu, Lys, Gly,
       or Ser.  The C-terminal Asp residue may be amidated.


<220>
<221>  misc_feature
<222>  (57)..(59)
<223>  Amino acid residues 50, 51, 57, 58 and 59 are Val, Val, Tyr, Met,
```

and Leu, respectively, in which zero or one to five of residues
50, 51, 57, 58, and 59 is substituted with Pro, Asp, Glu, Lys, Gly,
or Ser. The C-terminal Asp residue may be amidated.

<220>
<221> misc_feature
<222> (74)..(83)
<223> Amino acids 74-77 are all Lys or all Asp. Any one or all of
      78-83 can either be absent or present as Lys or Asp to form,
      in combination with residues 74-77, a C-terminal polylysine or
      polyaspartate segment of 4 to 10 residues in length.

<400> 44

Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Gly Gln Pro His Gly Gly
1   5   10   15

Gly Gly Trp Gly Gln Gly Gly Thr His Gly Gln Trp Asn Lys Pro Ser
            20   25   30

Lys Pro Lys Thr Asn Met Lys His Val Ala Gly Ala Ala Ala Ala Gly
        35   40   45

Ala Xaa Xaa Gly Gly Leu Gly Gly Xaa Xaa Xaa Gly Ser Ala Met Ser
    50   55   60

Arg Pro Leu Ile His Phe Gly Asn Asp Xaa Xaa Xaa Xaa Xaa Xaa Xaa
65   70   75   80

Xaa Xaa Xaa

<210> 45
<211> 146
<212> PRT
<213> Artificial Sequence

<220>
<223> synthetic

<220>
<221> misc_feature
<222> (1)..(10)
<223> Amino acid residues 7-10 are all Lys or all Asp. Any one or all
      of residues 1-6 can either be absent or present as Lys or Asp to
      form, in combination with residues 7-10, a N-terminal polylysine
      or polyaspartate segment of 4 to 10 residues in length.

<220>
<221> misc_feature
<222> (50)..(51)
<223> Amino acid residues 50, 51, 57, 58, 59 and residues 113, 114, 120,

121, 122 are the same and are Val, Val, Tyr, Met, and Leu,
respectively, in which zero or one to five of residues 50, 51, 57, 58,
and 59 and the same zero or one to five of residues 113, 114, 120,
121, and 122 is substituted with Pro, Asp, Glu, Lys, Gly or Ser.

<220>
<221> misc_feature
<222> (57)..(59)
<223> Amino acid residues 50, 51, 57, 58, 59 and residues 113, 114, 120,
121, 122 are the same and are Val, Val, Tyr, Met, and Leu,
respectively, in which zero or one to five of residues 50, 51, 57, 58,
and 59 and the same zero or one to five of residues 113, 114, 120,
121, and 122 is substituted with Pro, Asp, Glu, Lys, Gly or Ser.

<220>
<221> misc_feature
<222> (113)..(114)
<223> Amino acid residues 50, 51, 57, 58, 59 and residues 113, 114, 120,
121, 122 are the same and are Val, Val, Tyr, Met, and Leu,
respectively, in which zero or one to five of residues 50, 51, 57, 58,
and 59 and the same zero or one to five of residues 113, 114, 120,
121, and 122 is substituted with Pro, Asp, Glu, Lys, Gly or Ser.

<220>
<221> misc_feature
<222> (120)..(122)
<223> Amino acid residues 50, 51, 57, 58, 59 and residues 113, 114, 120,
121, 122 are the same and are Val, Val, Tyr, Met, and Leu,
respectively, in which zero or one to five of residues 50, 51, 57, 58,
and 59 and the same zero or one to five of residues 113, 114, 120,
121, and 122 is substituted with Pro, Asp, Glu, Lys, Gly or Ser.

<220>
<221> misc_feature
<222> (137)..(146)
<223> Amino acids 137-140 are all Lys or all Asp. Any one or all of
residues 140-146 can either be absent or present as Lys or Asp to
form, in combination with residues 137-140, a C-terminal polylysine
or polyaspartate segment of 4 to 10 residues in length.

<400> 45

Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Gly Gln Pro His Gly Gly
1   5   10   15

Gly Gly Trp Gly Gln Gly Gly Thr His Gly Gln Trp Asn Lys Pro Ser
        20   25   30

Lys Pro Lys Thr Asn Met Lys His Val Ala Gly Ala Ala Ala Ala Gly
        35   40   45

```
Ala Xaa Xaa Gly Gly Leu Gly Gly Xaa Xaa Xaa Gly Ser Ala Met Ser
    50              55              60

Arg Pro Leu Ile His Phe Gly Asn Asp Gly Gln Pro His Gly Gly Gly
65              70              75              80

Gly Trp Gly Gln Gly Gly Thr His Gly Gln Trp Asn Lys Pro Ser Lys
            85              90              95

Pro Lys Thr Asn Met Lys His Val Ala Gly Ala Ala Ala Ala Gly Ala
            100             105             110

Xaa Xaa Gly Gly Leu Gly Gly Xaa Xaa Xaa Gly Ser Ala Met Ser Arg
        115             120             125

Pro Leu Ile His Phe Gly Asn Asp Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa
    130             135              140

Xaa Xaa
145


<210>  46
<211>  37
<212>  PRT
<213>  Homo sapiens

<400>  46

Lys Cys Asn Thr Ala Thr Cys Ala Thr Gln Arg Leu Ala Asn Phe Leu
1               5               10              15

Val His Ser Ser Asn Asn Phe Gly Ala Ile Leu Ser Ser Thr Asn Val
            20              25              30

Gly Ser Asn Thr Tyr
        35


<210>  47
<211>  36
<212>  PRT
<213>  Mus sp.

<400>  47

Lys Cys Asn Thr Ala Thr Cys Ala Thr Gln Arg Leu Ala Asn Phe Val
1               5               10              15

Arg Ser Ser Asn Asn Leu Gly Pro Val Leu Pro Pro Thr Asn Val Gly
```

                 20   25   30

Ser Asn Thr Tyr
          35

<210> 48
<211> 47
<212> PRT
<213> Artificial Sequence

<220>
<223> synthetic

<220>
<221> misc_feature
<222> (1)..(10)
<223> Amino acid residues 7-10 either are present, together as all Lys
       or all Asp, or are all absent. When residues 7-10 are present then
       any one or all of residues 1-6 can either be absent or present
       as Lys or Asp to form, in combination with residues 7-10, a N-
       terminal polylysine or polyaspartate segment of 4 to10 residues in
       length.

<220>
<221> misc_feature
<222> (33)..(33)
<223> Amino acid residues 33, 36, and 37 are Phe, Ile and Leu,
       respectively, in which zero, one, two, or three of residues 33, 36 and
       37 is substituted with Pro, Asp, Glu, Lys, Gly, or Ser.

<220>
<221> misc_feature
<222> (36)..(37)
<223> Amino acid residues 33, 36, and 37 are Phe, Ile and Leu,
       respectively, in which zero, one, two, or three of residues 33, 36 and
       37 is substituted with Pro, Asp, Glu, Lys, Gly, or Ser.

<400> 48

Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Lys Cys Asn Thr Ala Thr
1   5   10   15

Cys Ala Thr Gln Arg Leu Ala Asn Phe Leu Val His Ser Ser Asn Asn
          20   25   30

Xaa Gly Ala Xaa Xaa Ser Ser Thr Asn Val Gly Ser Asn Thr Tyr
          35   40   45

<210> 49
<211> 84
<212> PRT
<213> Artificial Sequence

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<220>
<223>   synthetic

<220>
<221>   misc_feature
<222>   (1)..(10)
<223>   Amino acid residues 7-10 either are present, together as all Lys
        or all Asp, or are all absent.  When residues 7-10 are present then
        any one or all of residues 1-6 can either be absent or present
        as Lys or Asp to form, in combination with residues 7-10, a N-
        terrminal polylysine or polyaspartate segment of 4 to 10 residues in
        length.


<220>
<221>   misc_feature
<222>   (33)..(33)
<223>   Amino acid residues 33, 36, 37 and residues 70, 73, 74 are the same
        and are Phe, Ile, and Leu, respectively, in which zero, one, two,
        or three of residues 33, 36, and 37 and the same zero, one, two,
        or three of residues 70, 73, 74 is substituted with Pro, Asp,
        Glu, Lys, Gly, or Ser.


<220>
<221>   misc_feature
<222>   (36)..(37)
<223>   Amino acid residues 33, 36, 37 and residues 70, 73, 74 are the same
        and are Phe, Ile, and Leu, respectively, in which zero, one, two,
        or three of residues 33, 36, and 37 and the same zero, one, two,
        or three of residues 70, 73, 74 is substituted with Pro, Asp,
        Glu, Lys, Gly, or Ser.


<220>
<221>   misc_feature
<222>   (70)..(70)
<223>   Amino acid residues 33, 36, 37 and residues 70, 73, 74 are the same
        and are Phe, Ile, and Leu, respectively, in which zero, one, two,
        or three of residues 33, 36, and 37 and the same zero, one, two,
        or three of residues 70, 73, 74 is substituted with Pro, Asp,
        Glu, Lys, Gly, or Ser.


<220>
<221>   misc_feature
<222>   (73)..(74)
<223>   Amino acid residues 33, 36, 37 and residues 70, 73, 74 are the same
        and are Phe, Ile, and Leu, respectively, in which zero, one, two,
        or three of residues 33, 36, and 37 and the same zero, one, two,
        or three of residues 70, 73, 74 is substituted with Pro, Asp,
        Glu, Lys, Gly, or Ser.


<400>   49

Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Lys Cys Asn Thr Ala Thr
1               5                   10                  15


Cys Ala Thr Gln Arg Leu Ala Asn Phe Leu Val His Ser Ser Asn Asn
```

```
              20              25              30


Xaa Gly Ala Xaa Xaa Ser Ser Thr Asn Val Gly Ser Asn Thr Tyr Lys
        35              40              45


Cys Asn Thr Ala Thr Cys Ala Thr Gln Arg Leu Ala Asn Phe Leu Val
    50              55              60


His Ser Ser Asn Asn Xaa Gly Ala Xaa Xaa Ser Ser Thr Asn Val Gly
65              70              75              80


Ser Asn Thr Tyr
```

```
<210>  50
<211>  47
<212>  PRT
<213>  Artificial Sequence

<220>
<223>  synthetic

<220>
<221>  misc_feature
<222>  (23)..(23)
<223>  Amino acid residues 23, 26, and 27 are Phe, Ile, and Leu,
       respectively, in which zero, one, two or three of  residues 23, 26, and
       27 is substituted with Pro, Asp, Glu, Lys, Gly, or Ser.


<220>
<221>  misc_feature
<222>  (26)..(27)
<223>  Amino acid residues 23, 26, and 27 are Phe, Ile, and Leu,
       respectively, in which zero, one, two or three of residues 23, 26, and
       27 is substituted with Pro, Asp, Glu, Lys, Gly, or Ser.


<220>
<221>  misc_feature
<222>  (38)..(47)
<223>  Amino acid residues 38-41 are all Lys or all Asp, or are all absent.
       When all residues 38-41 are present, then any one or all of
       residues 42-47 can either be absent or present as Lys or Asp to
       form, in combination with residues 38-41, a C-terminal polylysine
       or polyaspartate segment of 4 to 10 residues in length.


<400>  50

Lys Cys Asn Thr Ala Thr Cys Ala Thr Gln Arg Leu Ala Asn Phe Leu
1               5               10              15


Val His Ser Ser Asn Asn Xaa Gly Ala Xaa Xaa Ser Ser Thr Asn Val
            20              25              30
```

```
Gly Ser Asn Thr Tyr Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa
        35                  40                  45
```

<210> 51
<211> 84
<212> PRT
<213> Artificial Sequence

<220>
<223> synthetic

<220>
<221> misc_feature
<222> (23)..(23)
<223> Amino acid residues 23, 26, 27 and residues 60, 63, 64 are the same
      and are Phe, Ile, and Leu, respectively, in which zero, one, two, or
      of residues 23, 26, and 27 and the same zero, one, two
      or three of residues 60, 63, 64 is substituted with Pro, Asp,
      Glu, Lys, Gly, or Ser.

<220>
<221> misc_feature
<222> (26)..(27)
<223> Amino acid residues 23, 26, 27 and residues 60, 63, 64 are the same
      and are Phe, Ile, and Leu, respectively, in which zero, one, two,
      or three of residues 23, 26, and 27 and the same zero, one, two,
      or three of residues 60, 63, 64 is substituted with Pro, Asp,
      Glu, Lys, Gly, or Ser.

<220>
<221> misc_feature
<222> (60)..(60)
<223> Amino acid residues 23, 26, 27 and residues 60, 63, 64 are the same
      and are Phe, Ile, and Leu, respectively, in which zero, one, two,
      or three of residues 23, 26, and 27 and the same zero, one, two,
      or three of residues 60, 63, 64 is substituted with Pro, Asp,
      Glu, Lys, Gly, or Ser.

<220>
<221> misc_feature
<222> (63)..(64)
<223> Amino acid residues 23, 26, 27 and residues 60, 63, 64 are the same
      and are Phe, Ile, and Leu, respectively, in which zero, one, two,
      or three of residues 23, 26, and 27 and the same zero, one, two,
      or three of residues 60, 63, 64 is substituted with Pro, Asp,
      Glu, Lys, Gly, or Ser.

<220>
<221> misc_feature
<222> (75)..(84)
<223> Amino acid residues 75-78 are all Lys or all Asp, or are all absent.
      When all residues 75-78 are present, then any one or all of
      residues 79-84 can either be Lys or Asp to form, in combination with
      residues 75-78, a C-terminal polylysine or polyaspartate segment

- 50 -

of 4 to 10 residues in length.

<400> 51

```
Lys Cys Asn Thr Ala Thr Cys Ala Thr Gln Arg Leu Ala Asn Phe Leu
1               5                   10                  15


Val His Ser Ser Asn Asn Xaa Gly Ala Xaa Xaa Ser Ser Thr Asn Val
            20                  25                  30


Gly Ser Asn Thr Tyr Lys Cys Asn Thr Ala Thr Cys Ala Thr Gln Arg
        35                  40                  45


Leu Ala Asn Phe Leu Val His Ser Ser Asn Asn Xaa Gly Ala Xaa Xaa
    50                  55                  60


Ser Ser Thr Asn Val Gly Ser Asn Thr Tyr Xaa Xaa Xaa Xaa Xaa Xaa
65                  70                  75                  80


Xaa Xaa Xaa Xaa
```

<210> 52
<211> 57
<212> PRT
<213> Artificial Sequence

<220>
<223> synthetic

<220>
<221> misc_feature
<222> (1)..(10)
<223> Amino acid residues 7-10 are all Lys or all Asp. Any one or all
      of residues 1-6 can either be absent or present as Lys or Asp to
      form, in combination with residues 7-10, a N-terminal polylysine
      or polyaspartate segment of 4 to 10 residues in length.

<220>
<221> misc_feature
<222> (33)..(33)
<223> Amino acid residues 33, 36, and 37 are Phe, Ile and Leu, respectively,
      in which zero, one, two, or three of residues 33, 36 and 37
      is substituted with Pro, Asp, Glu, Lys, Gly, or Ser.

<220>
<221> misc_feature
<222> (36)..(37)
<223> Amino acid residues 33, 36, and 37 are Phe, Ile and Leu, respectively,
      in which zero, one, two, or three of residues 33, 36 and 37
      is substituted with Pro, Asp, Glu, Lys, Gly, or Ser.

```
<220>
<221>  misc_feature
<222>  (48)..(69)
<223>  Amino acids 48-51 are all Lys or all Asp.  Any one or all of residues
       52-57 can either be absent or present as Lys or Asp to form,
       in combination with residues 66-69, a C-terminal polylysine or
       polyaspartate segment of 4 to 10 residues in length.


<400>  52

Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Lys Cys Asn Thr Ala Thr
1               5                   10                  15


Cys Ala Thr Gln Arg Leu Ala Asn Phe Leu Val His Ser Ser Asn Asn
            20                  25                  30


Xaa Gly Ala Xaa Xaa Ser Ser Thr Asn Val Gly Ser Asn Thr Tyr Xaa
            35                  40                  45


Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa
    50                  55



<210>  53
<211>  94
<212>  PRT
<213>  Artificial Sequence

<220>
<223>  synthetic

<220>
<221>  misc_feature
<222>  (1)..(10)
<223>  Amino acid residues 7-10 are all Lys or all Asp.  Any one or all
       of residues 1-6 can either be absent or present as Lys or Asp to
       form, in combination with residues 7-10, a N-terminal polylysine
       or polyaspartate segment of 4 to 10 residues in length.


<220>
<221>  misc_feature
<222>  (33)..(33)
<223>  Amino acid residues 33, 36, 37 and residues 70, 73, 74 are the same
       and are Phe, Ile, and Leu, respectively, in which zero, one, two,
       or three of residues 33, 36, and 37 and the same zero, one, two,
       or three of residues 70, 73, 74 is substituted with Pro, Asp,
       Glu, Lys, Gly, or Ser.


<220>
<221>  misc_feature
<222>  (36)..(37)
<223>  Amino acid residues 33, 36, 37 and residues 70, 73, 74 are the same
       and are Phe, Ile, and Leu, respectively, in which zero, one, two,
       or three of residues 33, 36, and 37 and the same zero, one, two,
```

or three of residues 70, 73, 74 is substituted with Pro, Asp,
Glu, Lys, Gly, or Ser.

<220>
<221> misc_feature
<222> (70)..(70)
<223> Amino acid residues 33, 36, 37 and residues 70, 73, 74 are the same
       and are Phe, Ile, and Leu, respectively, in which zero, one, two,
       or three of residues 33, 36, and 37 and the same zero, one, two,
       or three of residues 70, 73, 74 is substituted with Pro, Asp,
       Glu, Lys, Gly, or Ser.

<220>
<221> misc_feature
<222> (73)..(74)
<223> Amino acid residues 33, 36, 37 and residues 70, 73, 74 are the same
       and are Phe, Ile, and Leu, respectively, in which zero, one, two,
       or three of residues 33, 36, and 37 and the same zero, one, two,
       or three of residues 70, 73, 74 is substituted with Pro, Asp,
       Glu, Lys, Gly, or Ser.

<220>
<221> misc_feature
<222> (84)..(94)
<223> Amino acids 84-88 are all Lys or all Asp. Any one or all of residues
       89-94 can either be absent or present as Lys or Asp to form,
       in combination with residues 85-88, a C-terminal polylysine or
       polyaspartate segment of 4 to 10 residues in length.

<400> 53

Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Lys Cys Asn Thr Ala Thr
1   5   10   15

Cys Ala Thr Gln Arg Leu Ala Asn Phe Leu Val His Ser Ser Asn Asn
            20   25   30

Xaa Gly Ala Xaa Xaa Ser Ser Thr Asn Val Gly Ser Asn Thr Tyr Lys
        35   40   45

Cys Asn Thr Ala Thr Cys Ala Thr Gln Arg Leu Ala Asn Phe Leu Val
    50   55   60

His Ser Ser Asn Asn Xaa Gly Ala Xaa Xaa Ser Ser Thr Asn Val Gly
65   70   75   80

Ser Asn Thr Tyr Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa
            85   90

<210> 54
<211> 140

<212> PRT
<213> Homo sapiens

<400> 54

Met Asp Val Phe Met Lys Gly Leu Ser Lys Ala Lys Glu Gly Val Val
1   5   10   15

Ala Ala Ala Glu Lys Thr Lys Gln Gly Val Ala Glu Ala Ala Gly Lys
                20   25   30

Thr Lys Glu Gly Val Leu Tyr Val Gly Ser Lys Thr Lys Glu Gly Val
        35   40   45

Val His Gly Val Ala Thr Val Ala Glu Lys Thr Lys Glu Gln Val Thr
    50   55   60

Asn Val Gly Gly Ala Val Val Thr Gly Val Thr Ala Val Ala Gln Lys
65   70   75   80

Thr Val Glu Gly Ala Gly Ser Ile Ala Ala Ala Thr Gly Phe Val Lys
                85   90   95

Lys Asp Gln Leu Gly Lys Asn Glu Glu Gly Ala Pro Gln Glu Gly Ile
            100   105   110

Leu Glu Asp Met Pro Val Asp Pro Asp Asn Glu Ala Tyr Glu Met Pro
        115   120   125

Ser Glu Glu Gly Tyr Gln Asp Tyr Glu Pro Glu Ala
    130   135   140

<210> 55
<211> 140
<212> PRT
<213> Artificial Sequence

<220>
<223> synthetic

<220>
<221> misc_feature
<222> (37)..(37)
<223> One or more of the three sets of valine residues, represented as
      Xaa residue sets (1) 37 and 40; (2) 48, 49, and 52; and (3) 70,
      71, and 74, can be substituted with either all Glu, all Asp, all
      Pro, or all Lys.

<220>
<221> misc_feature
<222> (40)..(40)

<223> One or more of the three sets of valine residues, represented as
       Xaa residue sets (1) 37 and 40; (2) 48, 49, and 52; and (3) 70, 71,
       and 74, can be substituted with either all Glu, all Asp, all Pro,
       or all Lys.

<220>
<221> misc_feature
<222> (48)..(49)
<223> One or more of the three sets of valine residues, represented as
       Xaa residue sets (1) 37 and 40; (2) 48, 49, and 52; and (3) 70, 71,
       and 74, can be substituted with either all Glu, all Asp, all Pro,
       or all Lys.

<220>
<221> misc_feature
<222> (52)..(52)
<223> One or more of the three sets of valine residues, represented as
       Xaa residue sets (1) 37 and 40; (2) 48, 49, and 52; and (3) 70, 71,
       and 74, can be substituted with either all Glu, all Asp, all Pro,
       or all Lys.

<220>
<221> misc_feature
<222> (70)..(71)
<223> One or more of the three sets of valine residues, represented as
       Xaa residue sets (1) 37 and 40; (2) 48, 49, and 52; and (3) 70, 71,
       and 74, can be substituted with either all Glu, all Asp, all Pro,
       or all Lys.

<220>
<221> misc_feature
<222> (74)..(74)
<223> One or more of the three sets of valine residues, represented as
       Xaa residue sets (1) 37 and 40; (2) 48, 49, and 52; and (3) 70, 71,
       and 74, can be substituted with either all Glu, all Asp, all Pro,
       or all Lys.

<400> 55

Met Asp Val Phe Met Lys Gly Leu Ser Lys Ala Lys Glu Gly Val Val
1   5   10   15

Ala Ala Ala Glu Lys Thr Lys Gln Gly Val Ala Glu Ala Ala Gly Lys
            20   25   30

Thr Lys Glu Gly Xaa Leu Tyr Xaa Gly Ser Lys Thr Lys Glu Gly Xaa
        35   40   45

Xaa His Gly Xaa Ala Thr Val Ala Glu Lys Thr Lys Glu Gln Val Thr
    50   55   60

Asn Val Gly Gly Ala Xaa Xaa Thr Gly Xaa Thr Ala Val Ala Gln Lys

```
65                      70                      75                      80
```

Thr Val Glu Gly Ala Gly Ser Ile Ala Ala Ala Thr Gly Phe Val Lys
                 85   90   95

Lys Asp Gln Leu Gly Lys Asn Glu Glu Gly Ala Pro Gln Glu Gly Ile
             100   105   110

Leu Glu Asp Met Pro Val Asp Pro Asp Asn Glu Ala Tyr Glu Met Pro
             115   120   125

Ser Glu Glu Gly Tyr Gln Asp Tyr Glu Pro Glu Ala
    130   135   140